Myristica fragrans bio-active ester functionalized ZnO nanoparticles exhibit antibacterial and antibiofilm activities in clinical isolates

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A B S T R A C T

We provide a novel one-step/one-pot bio-inspired method of synthesis for Myristica fragrans leaf ester (MFLE) capped-zinc oxide nanoparticles (MFE-ZnONPs). Antibacterial and antibiofilm efficacies of MFE-ZnONPs were tested against the multi-drug resistant (MDR) Escherichia coli (E. coli-336), methicillin-resistant Staphylococcus aureus (MRSA-1) and methicillin-sensitive (MSSA-2) clinical isolates. Antibacterial screening using well diffusion assay revealed the cytotoxicity of ZnO-NPs in the range of 500-2000 μg/ml. MFE-ZnONPs significantly increased the zone of growth inhibition of E. coli-336 (17.0 ± 0.5 to 19.25 ± 1.0 mm), MSSA-2 (16.75 ± 0.8 to 19.0 ± 0.7 mm) and MRSA-1 (16.25 ± 1.0 to 18.25 ± 0.5 mm), respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) against E. coli-336, MRSA-1 and MSSA-2 were found to be 1500, 1000 and 500 μg/ml, and 2500, 2000 and 1500 μg/ml, respectively. A time and dose dependent reduction in the cell proliferation were also found at the respective MICs of tested strains. Scanning electron microscopy (SEM) of MFE-ZnONPs-treated strains exhibited cellular damage via loss of native rod and coccoid shapes because of the formation of pits and cavities. E. coli-336 and MRSA-1 strains at their MICs (1500 and 1000 μg/ml) sharply reduced the biofilm formation to 51% and 24%. The physico-chemical characterization via X-ray diffraction (XRD) ascertained the crystallinity and an average size of MFE-ZnONPs as 48.32 ± 2.5 nm. Gas chromatography-mass spectroscopy (GC–MS) analysis of MFE-ZnONPs unraveled the involvement of two bio-active esters (1) butyl 3-oxobut-2-yl ester and (2) α-monoolein) as surface capping/stabilizing agents. Fourier transform infrared (FTIR) analysis of MFLE and MFE-ZnONPs showed the association of amines, alkanes, aldehydes, amides, carbonyl and amines functional groups in the corona formation. Overall, our data provide novel insights on the rapid development of eco-friendly, cost-effective bio-synthesis of MFE-ZnONPs, showing their putative application as nano-antibiotics against MDR clinical isolates.

1. Introduction

Zinc oxide (ZnO) has a wide spectrum of applications owing to its intrinsic physico-chemical properties including large band gap (3.33 eV) and exciton energy (60 meV). Such unique characteristics have extended the entry of ZnO into next generation optoelectronics (Look et al., 1998; Tang et al., 1998) as well as application in ultra-violet (UV) lasers and light-emitting diodes (Aoki and Hatanaka, 2000; Pearton et al., 2004). Nanoscale metal and metal oxide particles possess tremendously large surface area and active sites allowing them to function as efficient catalysts (Shahbazali et al., 2014; Bhosale and Bhanage, 2015). Nano-sized ZnO structures also provide a strong foundation for their use in biomedical settings and the agriculture sector (Sangani et al., 2015; Hameed et al., 2016), amounting to global consumption of 10⁵ tons per year (Das et al., 2011). Furthermore, incorporation of ZnO-NPs into periodontal membranes can be used as a barrier for evading the colonization of Porphyromonas gingivalis, which is commonly involved in inducing periodontitis (Nasajpour et al., 2017).

Nanotechnologists have explored a diverse set of plant-based bio-inspired methods of NPs fabrication (Musarrat et al., 2015; Ali et al., 2015; Ali et al., 2016; Ali et al., 2018; Ali et al., 2019). During the last two decades, indigenous bio-actives present in the root, leaf and fruit

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Growth stimulation and alleviation of salinity stress to wheat by the biofilm forming *Bacillus pumilus* strain FAB10

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**Abstract**

Salinity ranks among the most severe environmental stressors that limit crop productivity. Use of microbial inoculants with desired traits is believed to be effective strategy to combat plant abiotic stress. Therefore, the objective was to isolate salt tolerant Bacillus spp. with multifunctional plant growth promoting traits including biofilm development and to evaluate its performance under salt stress conditions. We isolated and characterized a novel salt tolerant isolate of *Bacillus* sp. FAB10 with multifunctional traits by screening of 56 selected Bacillus isolates from rhizospheric soils. The isolate FAB10 was identified as *B. pumilus* based on 16S rRNA gene sequence analysis. The FAB10 isolate produced strong biofilm, enhanced amount of exopolysaccharides, IAA, ACC-deaminase activity and solubilized phosphate in vitro. The isolate FAB10 forms biofilm and expressed its associated traits at the different NaCl concentrations in vitro. Biofilm development on Wheat root surface was also demonstrated at 250mM salt (NaCl) concentration. Successful root colonization by the FAB10 isolate was demonstrated via scanning electron microscopy and viable counts. Wheat plant var. 343 inoculated with *Bacillus pumilus* isolate FAB10 grown in the presence of different concentrations (0 to 250mM) of NaCl under pot house conditions. At elevated concentration of NaCl adverse effect on wheat growth parameters and other biochemical attributes pertaining to photosynthesis, transpiration, and content of proline in plant tissue was recorded in uninoculated plants. However, inoculated plants showed a significant (*p* < 0.005) improvement in most of the above parameters. Similarly a significant (*p* < 0.005) reduction in antioxidant enzyme activities (catalase, superoxide dismutase, and glutathione reductase) and malonaldehyde content in wheat was observed in FAB10 inoculated plants than uninoculated plants in the presence of salt.

The findings indicated that multifunctional traits of the FAB10 contribute to NaCl stress alleviation in wheat plants through multiple mode of action and it could be exploited under field condition.

1. Introduction

Wheat (*Triticum aestivum*) is a vital staple food for billions people of the globe. As a consequence of continued increases in human population of the global demand for wheat is expected to reach about 536–551 million tons within a decade (Van Bavel, 2013).

Soil salinity mainly due to NaCl of agricultural lands is considered one of the major threats to crop productivity including wheat. At high level of salinity, accumulation of Na ions results changes in the soil physicochemical characteristics such as decrease in soil porosity, soil aeration and water conductance and this in turn affect plant nutrient uptake from soil and overall soil health (Hmaeid et al., 2018; Izadi et al., 2014). Adverse effects of salinity on the growth characteristics of the wheat such as panicle initiation, number of tiller, formation of spikelet and grain size, and delayed heading and physiological attributes have been well documented (Negrão et al., 2017).

Stressed agricultural soils also adversely influence soil microbial biome (Vimal et al., 2017). However plant under stress conditions can

**Abbreviations:** ACC, 1-aminocyclopropane-1-carboxylate; CSH, cell surface hydrophobicity; CI, CO₂ concentration; E, transpiration rate; gₛ, stomatal conductance; HCN, hydrogen cyanide; IAA, indole acetic acid; MATH, microbial adhesion to hydrocarbons; PN, net photosynthesis rate; PGPR, plant growth promoting rhizobacteria

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Bioactive extracts of *Carum copticum* L. enhances efficacy of ciprofloxacin against MDR enteric bacteria

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1. Introduction

The widespread occurrence of extended spectrum β-lactamases (ESβLs) producing enteric bacteria and their co-resistance with fluoroquinolones has impaired the current antimicrobial therapy. This has prompted the search for new alternatives through synergistic approaches with herbal extracts. In this study *Carum copticum* (seeds) was extracted first in methanol and then subsequently extracted in different organic solvents. MIC of plant extracts, ciprofloxacin and thymol was determined by broth microdilution method using TTC. Synergism between plant extracts and ciprofloxacin was assayed by the checkerboard method. Chemical constituents of active extracts were analyzed by GC-MS. Methanolic, hexane and ether extract of *Carum copticum* exhibited significant antibacterial activity with MIC values ranging from 0.25 mg/ml to 2.0 mg/ml. Synergy analysis between *Carum copticum* extracts and ciprofloxacin combinations revealed FIC index in the range of 0.093–0.25. About 81% ciprofloxacin resistant ESβL producing enteric bacteria were re-sensitized in the presence of 15.6–250 μg/ml of methanolic extract of *Carum copticum*. Moreover, ciprofloxacin showed 8 to 64 folds reduction in MIC in presence of 250 and 500 μg/ml of hexane extract. Whereas, 4–32 folds reduction in MIC of ciprofloxacin was achieved in the presence of 31.25 and 62.5 μg/ml of ether extract, indicating synergistic enhancement of drug activity. The chemical analysis of hexane and ether extracts by GC-MS revealed the common occurrence of one or more phenolic hydroxyl at different locations on benzene ring. This study demonstrated the potential use of herbal extract of *Carum copticum* in combination therapy against ESβL producing bacteria.

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New tailored substituted benzothiazole Schiff base Cu(II)/Zn(II) antitumor drug entities: effect of substituents on DNA binding profile, antimicrobial and cytotoxic activity

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New tailored Cu(II) & Zn(II) metal-based antitumor drug entities were synthesized from substituted benzothiazole o-vanillin Schiff base ligands. The complexes were thoroughly characterized by elemental analysis, spectroscopic studies {IR, 1H & 13C NMR, ESI–MS, EPR} and magnetic susceptibility measurements. The structure activity relationship (SAR) studies of benzoazole Cu(II) & Zn(II) complexes having molecular formulas [C30H20Cl2CuN5O7S2], [C30H20Cl2CuF2N5O7S2], [C30H20CuCl2N4O4S2Zn], [C30H20Cl2N4O4S2Zn], and [C30H20F2N5O7S2Zn], with CT–DNA were performed by employing absorption, emission titrations, and hydrodynamic measurements. The DNA binding affinity was quantified by Kb and Ksv values which gave higher binding propensity for chloro-substituted Cu(II) [C30H20Cl2CuN5O7S2] complex, suggestive of groove binding mode with subtle partial intercalation. Molecular properties and drug likeness profile were assessed for the ligands and all the Lipinski’s rules were found to be obeyed. The antimicrobial potential of ligands and their Cu(II) & Zn(II) complexes were screened against some notably important pathogens viz., E. coli, S. aureus, P. aeruginosa, B. subtilis, and C. albicans. The cytotoxicity of the complexes [C30H20Cl2CuN5O7S2], [C30H20CuCl2N4O4S2Zn], [C30H20Cl2N4O4S2Zn], and [C30H20F2N5O7S2Zn] were evaluated against five human cancer cell lines viz., MCF–7 (breast), MIA–PA–CA–2 (pancreatic), HeLa (cervix) and Hep–G2 (Hepatoma) and A498 (Kidney) by SRB assay which revealed that chloro-substituted [C30H20Cl2CuN5O7S2] complex, exhibited pronounced specific cytotoxicity with GI50 value of 4.8 μg/ml against HeLa cell line. Molecular docking studies were also performed to explore the binding modes and orientation of the complexes in the DNA helix.

Keywords: Benzothiazole Cu(II) & Zn(II) Schiff base complexes; structure activity relationship; antimicrobial studies; cytotoxicity; molecular docking

1. Introduction

N–heterocycles viz., benimidazole, benzothiazole, indole, pyrazole, quinolines, etc., have emerged as an important distinct class of anticancer therapeutic agents (Kumar & Kumar, 2016). These compounds possess N–heterocyclic aromatic pharmacophore synthon which is responsible for harnessing medicinal properties by multiple mechanisms which may be largely responsible for inhibiting cell growth and inducing apoptosis (Solomon, Hu, & Lee, 2009). FDA’s Center for Drug Evaluation and Research (CDER) approves a wide range of new molecular entities (NMEs) possessing bioactive moieties which may provide important new therapies for treating patients with many chronic diseases. Many prominent N–heterocyclic compounds developed by reputed pharmaceutical companies (Pfizer, AstraZeneca, Novartis, etc.) have been approved by US FDA as anticancer agents for myriad phenotypes of cancers (http://www.fda.gov). Unfortunately, many of these NMEs fail either in clinical trials or in the developmental stages due to issues of systemic toxicity and low absorption at cellular levels.

Among the N–heterocycles, benzothiazole derivatives have gained prominence due to diverse biological activities viz., antimicrobial, antiviral, and anticancer activities (Racané et al., 2013). Besides this, benzothiazole is a key component of nucleic acids, and therefore, can participate directly in encoding of genetic information. Being an important drug synthon, it can be tethered to other organic ancillary ligands for targeted therapy to yield more efficacious and potent ligand scaffolds. Several benzothiazole derivatives are reported to possess excellent in vitro and in vivo cytotoxicity at low nanomolar concentrations (Prota et al., 2014). Literature reports reveal that 2-aminobenzothiazole derivatives were synthesized and tested at National Cancer Institute (NCI) against nine panels of cancer cell lines for antitumor activity. It was observed that chloro derivative, 7-chloro–N-(2,6-dichlorophenyl)benz[d]thiazol–2-amine was most active against non-small lung cancer cell

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Potential of Nanoparticles in Combating Candida Infections

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Abstract: Aims: The aim of this review is to survey the recent progress made in developing the nanoparticles as antifungal agents especially the nano-based formulations being exploited for the management of Candida infections.

Discussion: In the last few decades, there has been many-fold increase in fungal infections including candidiasis due to the increased number of immunocompromised patients worldwide. The efficacy of available antifungal drugs is limited due to its associated toxicity and drug resistance in clinical strains. The recent advancements in nanobiotechnology have opened a new hope for the development of novel formulations with enhanced therapeutic efficacy, improved drug delivery and low toxicity.

Conclusion: Metal nanoparticles have shown to possess promising in vitro antifungal activities and could be effectively used for enhanced and targeted delivery of conventionally used drugs. The synergistic interaction between nanoparticles and various antifungal agents have also been reported with enhanced antifungal activity.

Keywords: Antifungal, Candida albicans, candidiasis, nanoparticles, synergy, nanobiotechnology.

1. INTRODUCTION

There has been a tremendous increase in fungal infections over the last few decades globally, resulting in significant mortality and morbidity rate, specially in immunocompromised patients [1]. Fungal infections can be classified as (i) subcutaneous, (ii) superficial and (iii) systemic mycoses. The most prevalent fungal agents causing such infections are Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus [2]. Nowadays, drug discovery research to combat fungal infections is focussed (i) to understand the mechanism of pathogenicity at molecular level, (ii) factors responsible for the development of resistance and (iii) to develop novel therapeutic agents [3]. Among human pathogenic fungi, Candida spp, especially Candida albicans has emerged as one of the most common opportunistic pathogens. High mortality associated with this fungus is exceeding up to 30% which is mainly due to the limited availability of antifungal drugs and the development of drug resistance [4, 5]. The antifungal drugs available to combat fungal infections mainly include azoles, polyenes, alkylamines and echinocandins [6]. Structures of most commonly used antifungal drugs discussed in the article are presented in Fig. (1). Fluconazole is one of the most commonly used antifungal drug to treat C. albicans infections due to its low toxicity and high bioavailability [7, 8]. It exhibits high bioavailability profile with more than 80% of orally administered drug used in chemotherapy and fairly (70%) excreted from the human body and less than 10% of it is found in bound form with serum protein. [9]. The emergence of fungal strains being resistant to azoles is due to its unmonitored and excessive use [10]. C. albicans has developed resistance mechanism not only to fluconazole but also cross-resistance to other antifungal drugs like amphotericin B and nystatin [11]. In addition to specific drug resistance mechanism, several hundred Candida spp form extensive biofilms in the process of infection and become tolerant of antifungal drugs several hundred folds, making eradication more difficult [11-13]. Development of biofilms results in poor penetration of conventional drugs and it makes eradication of such infections very difficult.

Despite remarkable progress in understanding the molecular mechanism of C. albicans virulence, pathogenicity, biofilms [11] and diagnostic methods [14]; there is little success in combating the fungal infections caused by drug-resistant strains of C. albicans. Therefore, development of improved antifungal therapy against candidiasis is a need of hour. Several researchers have attempted various approaches such as combination or synergistic therapy, antipathogenic and anti-biofilm strategy in combination with other antifungal agents have achieved limited success specially in systemic infection [14, 15].
Fluorescent *Pseudomonas*-FAP2 and *Bacillus licheniformis* interact positively in biofilm mode enhancing plant growth and photosynthetic attributes

Firoz Ahmad Ansari & Iqbal Ahmad

Compatible interaction between commonly used plant growth promoting rhizobacteria (PGPR) in biofilm mode *in vitro* and in the rhizosphere is expected to provide better understanding for the development of effective consortium. With the above hypothesis, the present study evaluated two characterized PGPR (*Pseudomonas fluorescens* FAP2 and *Bacillus licheniformis* B642) for their biofilm-related functions using standard protocols. The interaction between the FAP2 and B642 in planktonic mode was studied by plate spot/overlay method and competitive growth assessment. Biofilm development on a microtitre plate and a glass surface was studied by standard methods. Biofilm formation was characterized by SEM. Rhizosphere and rhizoplane colonization of wheat seedlings by both isolates individually and by co-inoculation was studied by determining CFU/g of soil/root samples. Biofilm development on the root surface was further analyzed by SEM. Both isolates demonstrated multiple plant growth promoting (PGP) traits (production of IAA, siderophore, and ammonia; phosphate solubilization) and biofilm-related functions such as production of EPS, alginate, cell surface hydrophobicity and swarming motility. Both strains formed strong biofilms on a glass cover slip *in vitro*. Interaction between the two strains under the planktonic mode revealed no antagonism in terms of growth inhibition and competitive growth kinetics. Similarly, FAP2 and B642 strains formed a mixed biofilm on a glass cover slip as well as on seedling roots. Wheat rhizosphere and rhizoplane were colonized by both isolates as evidenced from their viable counts in single and co-inoculation. The effect of single and co inoculation revealed the significant enhancement of vegetative growth and photosynthetic parameters such as chlorophyll content, transpiration rate (E), internal CO₂ concentration (Ci), stomatal conductance (gs), and net photosynthetic rate (Pₙ) and leaf water potential (LWP) as compared to uninoculated control. Indigenous *Pseudomonas fluorescens* FAP2 strain and *Bacillus licheniformis* B642 are compatible PGPR in both planktonic and biofilm modes of growth and therefore could be developed effective consortium of PGPR. Further indepth investigation is required to understand molecular mechanism of the interaction in biofilm mode of growth under natural condition.

Soil microbial diversity and their functions are of prime importance to soil fertility. The rich diversity of soil microbe and microbial dynamics are maintained through multispecies interactions in both planktonic and biofilm modes. It has been reported that environmental biofilms comprise the predominant mode of growth for bacteria, and soil surfaces provide for establishment of multispecies biofilms. The biofilm mode of growth provides bacteria with protection against harsh environmental conditions and increased survivability by altering modes of growth and gene expression. Biofilms are also known to impart a several-fold increased tolerance to antibiotics, toxic chemicals and desiccation. Different bacteria differ in their ability to develop biofilms *in vitro* and *in situ*. Interaction between two organisms may be positive, negative, or neutral. It is believed that beneficial
Interference of phosphane copper (I) complexes of β-carboline with quorum sensing regulated virulence functions and biofilm in foodborne pathogenic bacteria: A first report

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Foodborne pathogens are one of the major cause of food-related diseases and food poisoning. Bacterial biofilms and quorum sensing (QS) mechanism of cell–cell communication have also been found to be associated with several outbreaks of foodborne diseases and are great threat to food safety. Therefore, in the present study, we investigated the activity of three tetrahedrally coordinated copper(I) complexes against quorum sensing and biofilms of foodborne bacteria. All the three complexes demonstrated similar antimicrobial properties against the selected pathogens. Concentration below the MIC i.e. at sub-MICs all the three complexes interfered significantly with the quorum sensing regulated functions in C. violaceum (violacein), P. aeruginosa (elastase, pyocyanin and alginate production) and S. marcescens (prodigiosin). The complexes demonstrated potent broad-spectrum biofilm inhibition in Pseudomonas aeruginosa, E. coli, Chromobacterium violaceum, Serratia marcescens, Klebsiella pneumoniae and Listeria monocytogenes. Biofilm inhibition was visualized using SEM and CLSM images. Action of the copper(I) complexes on two key QS regulated functions contributing to biofilm formation i.e. EPS production and swarming motility was also studied and statistically significant reduction was recorded. These results could form the basis for development of safe anti-QS and anti-biofilm agents that can be utilized in the food industry as well as healthcare sector to prevent food-associated diseases.

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1. Introduction

Foodborne pathogenic bacteria are the most frequent cause found associated with the foodborne diseases and food poisoning and hence pose a potential danger to both the food safety and human health (Oliver et al., 2005). There are several types of bacteria implicated for contamination of raw and processed food items such as E. coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa, Serratia, Clostridium and Listeria monocytogenes (Zhao et al., 2017). In recent times, significant morbidity and mortality is observed because of the foodborne diseases making it a serious public health problem (Zhao et al., 2016). Foodborne bacteria not only threaten human health but also cause enormous economic losses to the food industry (Zhao et al., 2014; Zhao et al., 2017). The food industry has been facing a major problem of food spoilage due to the biofilm formation that has been found responsible for various outbreaks of foodborne infection (Aarnisalo et al., 2007). Biofilms are the hydrated matrix of extracellular polymeric substances produced by bacteria that adhere them to the surface help-
Recent Progress in Metal-Microbe Interactions: Prospects in Bioremediation

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Abstract

Heavy metal contamination due to natural and anthropogenic source is a major environmental problem. Release of metal from use of agrochemicals, industrial effluents and wastewater residues and their accumulation in food causes serious dilemma to animal and human health concern. On the other hand microbial population gets affected from metal toxicity at elevated concentration. With the result microbes develops various resistance mechanism to cope with metal toxicity. Both physiological and genetic mechanisms are involved in developing such resistance. Recent advances on metal-bacteria interaction has led to better understanding of metal accumulation/detoxification or biotransformation and bioremediation of metals through application of such bacteria. Role of various transport protein families involved in heavy metal metabolism are now explored. This article provides insights of metal-bacteria interaction in terms of resistance mechanisms and role of various transport proteins and its potential application in bioremediation of metal pollution.

Keywords: Heavy metal resistance; bioremediation; public health; efflux pump; genes. Transport proteins.
Broad-spectrum quorum sensing and biofilm inhibition by green tea against gram-negative pathogenic bacteria: Deciphering the role of phytocompounds through molecular modelling

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ABSTRACT

The emerging prevalence of multidrug-resistance in Gram-negative pathogens, due to conventional antimicrobial therapeutics, has led the researchers to emphasize on development of alternative novel strategies to suppress the bacterial virulence and pathogenicity through inhibition of quorum sensing (QS) and biofilms. QS is a bacterial communication system to produce density-dependent response via chemical signalling that controls pathogenesis and biofilms formation. Leaves of green tea are used worldwide as beverage which is also known for its broad-spectrum therapeutic efficacy. In this work, we have identified and characterized the most bioactive fraction of green tea extract and evaluated the anti-QS and antibiofilm activity of green tea ethyl acetate fraction (GTEF) i.e. most active fraction, on three different Gram-negative bacterial pathogens. GTEF inhibited the violacein production by > 75% in C. violaceum 12472. Many virulence factors of P. aeruginosa PAO1 viz. pyocyanin, pyoverdin, exoprotease, elastase, rhamnolipid production, and swimming motility were remarkably reduced in presence of sub-MICs of GTEF. Moreover, prodigiosin, protease activity, cell surface hydrophobicity, and swimming of S. marcescens MTCC 97 were also decreased significantly by the supplementation of GTEF in culture media. GTEF exhibited broad-spectrum antibiofilm action with > 80% reduction in biofilm formation of test pathogens. In silico studies gave a mechanistic insight of action of GTEF. Molecular modelling revealed that phytoconstituents detected by GC/MS exhibited affinity (in order of 10^{4} M^{-1}) towards AHL synthases (LasI and EsaI). The molecular binding between phytocompounds and receptor proteins (LasR, RhlR, and PqsR) of QS circuit was also energetically favourable (ΔG° ≥ 5.0 kcal mol^{-1}) and supported by hydrogen bonds and hydrophobic interactions. These compounds were found to be docked in ligand binding domain of CviR and occupied same cavity as that of its antagonist. Squalene and thunbergol interacted with LasA at tartaric acid binding pocket and the complex was strengthened with binding energy −5.9 kcal mol^{-1}. Moreover, interaction of thunbergol with biofilm-associated proteins viz. PilT and PilY1, might be disabling the pilus assembly and consequently inhibiting biofilm formation. In vivo validation of results suggested the protective role GTEF against QS-mediated pathogenicity and it might become a novel non-antibiotic QS inhibitor to control bacterial infection.

1. Introduction

The worldwide emergence of multidrug resistance in bacterial pathogens has created a global health issue. The demand can only be met by the development of novel alternative strategies to overcome microbial infections. The antibiotics can no longer be entrusted for long-term therapeutic applications either due the development of resistance against them or detrimental effects of on host’s microbiome or both [1]. In recent past, research on discovery or synthesis of anti-infective agents have focussed on selective intervention of virulence pathways to manage or cure microbial infections as such drug targets do not affect the survival of pathogens making them less prone to develop resistance compared to conventional antimicrobials [2]. To be developed as next generation anti-infective drugs, bioactive compounds derived from natural sources, such as medicinal plants, are exhibiting promising therapeutic properties in management of evolving resistance in pathogens [3].

Quorum sensing (QS) is a communication system in bacteria to give density dependent response via chemical signalling that includes pathogenesis and biofilms formation in many pathogenic bacteria [4]. The

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Research Article

Antibacterial Effect of Silver Nanoparticles Synthesized Using Murraya koenigii (L.) against Multidrug-Resistant Pathogens

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Development of multidrug resistance among pathogens has become a global problem for chemotherapy of bacterial infections. Extended-spectrum β-lactamase-(ESβL-) producing enteric bacteria and methicillin-resistant Staphylococcus aureus (MRSA) are the two major groups of problematic MDR bacteria that have evolved rapidly in the recent past. In this study, the aqueous extract of Murraya koenigii leaves was used for synthesis of silver nanoparticles. The synthesized MK-AgNPs were characterized using UV-vis spectroscopy, FTIR, XRD, SEM, and TEM, and their antibacterial potential was evaluated on multiple ESβL-producing enteric bacteria and MRSA. The nanoparticles were predominantly found to be spheroidal with particle size distribution in the range of 5–20nm. There was 60.86% silver content in MK-AgNPs. Evaluation of antibacterial activity by the disc-diffusion assay revealed that MK-AgNPs effectively inhibited the growth of test pathogens with varying sized zones of inhibition. The MICs of MK-AgNPs against both MRSA and methicillin-sensitive S. aureus (MSSA) strains were 32 μg/ml, while for ESβL-producing E. coli, it ranged from 32 to 64 μg/ml. The control strain of E. coli (ECS) was relatively more sensitive with an MIC of 16 μg/ml. The MBCs were in accordance with the respective MICs. Analysis of growth kinetics revealed that the growth of all tested S. aureus strains was inhibited (~90%) in presence of 32 μg/ml of MK-AgNPs. The sensitive strain of E. coli (ECS) showed least resistance to MK-AgNPs with >81% inhibition at 16 μg/ml. The present investigation revealed an encouraging result on *in vitro* efficacy of green synthesized MK-AgNPs and needed further *in vivo* assessment for its therapeutic efficacy against MDR bacteria.

1. Introduction

Development of multidrug resistance has become a global issue with serious consequences in the management of infectious diseases caused by pathogenic bacteria [1]. This is mainly due to indiscriminating use of antibiotics in human healthcare, agriculture, and veterinary medicine [2]. The most common problematic multidrug-resistant pathogens are Acinetobacter baumannii, ESβL-producing E. coli, penicillin-resistant Streptococcus pneumoniae, Klebsiella pneumoniae, vancomycin-resistant Enterococcus, methicillin-resistant S. aureus, and extensively drug-resistant *Mycobacterium tuberculosis* [3]. ESβL groups of β-lactamases which are evolving at an alarming rate have ability to hydrolyse third-generation cephalosporins in addition to aztreonam [4, 5]. Methicillin-resistant *S. aureus* (MRSA) is the paramount cause of nosocomial infections associated with pneumonia, bloodstream infections, and surgical site infections [6]. Considering these problems,
Biosorbing Potentials of *Pseudomonas aeruginosa* SFP1 to Combat Cr(VI) Stress in *Cicer Arietinum* Seedlings

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ABSTRACT

Hexavalent chromium among metal pollutants is a major threat due to its mutagenic and carcinogenic impacts. Considering these, bacterial strain SFP1 was isolated from metal polluted soil (identified as *Pseudomonas aeruginosa* using 16SrRNA gene sequencing) showed significant tolerance to Cr (VI) and displayed chromium reducing ability under variable environmental conditions. The dried biomass of SFP1 adsorbed chromium maximally at pH 6 and 30±2°C which decreased consistently with increase in Cr concentration. The values obtained for chromium sorption by strain SFP1 using both Langmuir (R²=0.992) and Freundlich isotherms (R²=0.999) were strongly and positively correlated. The surface functional groups of dried biomass detected by Fourier transform infrared (FTIR) spectroscopy were amino, carboxyl, hydroxyl, and carbonyl groups. Also SEM-EDX revealed significant deposition of Cr and modification of bacterial cells after Cr(VI) exposure. The chickpea seeds primed with SFP1 strain displayed enhanced germination compared with metal treated but uninoculated plants. The present study suggests that the bacteria removes chromium efficiently and hence, could be used for the management of industrial wastes and other environmental contaminants.

**Keywords:** Chromium (VI) toxicity, Biosorption, *Pseudomonas aeruginosa*, Chickpea-Germination

1. Introduction:

Among heavy metals, chromium has been described as a priority pollutant by US EPA and is considered carcinogenic. Among different oxidation states, the trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)) has been reported to be approximately 100 times more toxic [1] and 1000 times more mutagenic than Cr(III) [2]. Conventionally, different physico-chemical methods like precipitation, electrochemical treatment and ion exchange have been used to remediate metal polluted environment. However, these methods are expensive, environmentally unfriendly and produce residues which are even more toxic than the parent metals. Due to these problems, the bioremediation approach especially the use of microorganisms is regarded as an inexpensive and environmentally safe strategy that offers the possibility to destroy toxic chromium to harmless forms [3,4]. Biosorption involving bacteria is an efficient method employed for the removal of Cr (VI) from industrial effluents [5] which decrease the concentration of chromium ions in solution. Realizing the chromium threat and importance of microbes in toxicity abatement on the other hand, the present study was aimed at identifying bacteria capable of biosorbing chromium under different conditions and used to assess the bioremediation potential using chickpea (*Cicer arietinum* L.)

2. Materials and methods

2.1 Isolation and screening of chromium tolerant bacteria

The bacterial strain SFP1 was isolated from rhizosphere grown in metal polluted fields of Unnao (26°32’25.0”N 80°29’14.3”E), UP India.

The ability of bacterial strains to grow under increasing concentrations of chromium was tested both on solid agar plate and in liquid culture medium. For this, bacterial strains were aseptically streaked on nutrient agar plates supplemented with 100–2000 µg/ml potassium dichromate, and checked for growth after incubation at 30±2°C for 48 h. The bacterial strain SFP1 exhibiting the highest tolerance to Cr (VI) was selected for further studies. Chromium containing NB (0-100µg/ml) was inoculated with overnight grown cultures and incubated at 30±2°C for 4 days under continuous shaking (120 rpm) in a rotary shaker. Further SEM micrographs of both untreated and Cr(VI) treated cells was observed for morphological alterations while EDX analysis was carried to determine the metal deposition.

2.2 Molecular identification and phylogenetic tree of metal tolerant strain SFP1

The chromium tolerant strain SFP1 was selected and characterized by standard morphological and biochemical methods [6]. The nucleotide sequence of strain SFP1 was analysed commercially by Macrogen Inc., Seoul, South Korea using the 16S rRNA genes) involving universal primers, 785F (GGATTAGATACCCTGGTA) and 907R (CCGTCAATTCTTTRAGTTT). The sequence (859 bp) so obtained were analyzed by adopting BLASTn tool (http://www.ncbi.nlm.nih.gov/BLAST) to accurately identify and match the sequence of isolates with the nearest neighbour sequence obtainable at the NCBI database. All the sequence were aligned using Clustal W and the aligned data was used for phylogenetic analysis using MEGA7 by neighbour-joining method with 1000 boot strap replicates.
Kitazin-pea interaction: understanding the fungicide induced nodule alteration, cytotoxicity, oxidative damage and toxicity alleviation by *Rhizobium leguminosarum*

Mohammad Shahid, Mohammad Saghir Khan and Murugan Kumar

Realizing the severity of fungicidal toxicity to legumes and importance of fungicide tolerant rhizobia in legume production, kitazin tolerant (2400 μg mL⁻¹) strain RP1 was recovered from pea nodules and was identified as *Rhizobium leguminosarum* (accession no. KY940047). *R. leguminosarum* produced indole acetic acid (80.5 ± 2.5 mL⁻¹), siderophores: salicylic acid (54 ± 7.3 μg mL⁻¹) and 2,3-dihydroxybenzoic acid (31.9 ± 2.7 μg mL⁻¹), α-ketobutyrate (51 ± 3.2 mg per mg per protein per hour), solubilized insoluble phosphate (29.5 ± 18 μg mL⁻¹) and secreted 29.5 + 2.6 μg mL⁻¹ exopolysaccharides, which, however, decreased consistently with gradually increasing kitazin concentrations. Beyond the tolerance level, kitazin caused structural damage and altered membrane integrity of RP1, as revealed under scanning (SEM) and confocal (CLSM) electron microscopy. Phytotoxicity of kitazin to peas was obvious under both *in vitro* and *in vivo* conditions. A significant reduction of 23, 68, 57 and 50% in germination, seedling vigor index, plumule length and radicle length was found at 2x kitazin compared to the control. Cellular damage and cytotoxicity induced by kitazin in membrane altered root cells was detected with acridine orange/propidium iodide (AO/PI) and Evans blue dye. A maximum increase of 1.72, 5.2, 9.3 and 1.72, 5.2, 9.3-fold in red and blue fluorescence was quantified at 1x, 2x, and 3x doses of kitazin, respectively. In contrast, application of *R. leguminosarum* RP1 alleviated toxicity and enhanced the length of plant organs, dry biomass, symbiotic attributes, photosynthetic pigments, nutrient uptake and grain features of peas comparatively uninoculated and fungicide-treated plants. Additionally, strain RP1 expressively reduced the antioxidant enzymes peroxidase, ascorbate peroxidase, guaiacol peroxidase, catalase and proline and antioxidant enzymes. So, it can safely be suggested to legume growers that RP1 strain could significantly increase the performance of peas while reducing the levels of endogenous antioxidative enzymes.

Due to their high nutritive value, legumes are considered important food crops for human beings and have habitually been cultivated around the world since primeval times. But, fungal diseases cause huge losses to legume production globally. To overcome such losses, legume growers adopt common and general practices such as pre-sowing application of fungicides. The irregular and injudicious application of such plant protectants has, however, been found harmful to microbial diversity, soil fertility and legume production. Numerous literature reports on the toxic and harmful action of synthetic fungicides on soil properties and legume crops are available. Among them are a destruction of soil fecundity that leads to losses in growth, symbiosism and yield. Some fungicides obliterate nodule formation, affect biological nitrogen fixation (BNF), decrease the formation of photosynthetic and carotenoid pigments and disturb the whole physiological machinery of plant by inhibiting electron transport systems (ETS) of...
Comparative in situ ROS mediated killing of bacteria with bulk analogue, *Eucalyptus* leaf extract (ELE)-capped and bare surface copper oxide nanoparticles

Khursheed Ali, Bilal Ahmed, Sabiha M. Ansari, Quaiser Saquib, Abdulaziz A. Al-Khedhairi, Sourabh Dwivedi, Majed Alshaerid, Mohd Saghir Khan, Javed Musarrat

**A R T I C L E  I N F O**

Keywords:
- Biomoinspired CuONPs
- Terpenoids
- GC–MS
- Flow cytometry
- ROS
- Green nanoparticles

**A B S T R A C T**

This study demonstrates a simple one-pot green method for biosynthesis of terpenoids encapsulated copper oxide nanoparticles (CuONPs) using aqueous leaf extract of *Eucalyptus globulus* (ELE), as reducing, dispersing, and stabilizing agent. Indeed, the greater attachment and internalization of ELE-CuONPs in Gram-positive and -negative biofilm producing clinical bacterial isolates validated the hypothesis that terpenoids encapsulated CuONPs are more stable and effective antibacterial and antifilm agent vis-a-vis commercially available nano and micro sized analogues. Gas chromatography-mass spectroscopy (GC–MS) analysis of pristine ELE identified 17 types of terpenoids based on their mass-to-charge (m/z) ratios. Amongst them four bioactive terpenoids viz. terpineols, 2,6-octadienal-3,7-dimethyl, benzamidophenyl-4-benzoate and β-eudesmol were found associated with the CuONPs as ELE-cap, and most likely involved in the nucleation and stabilization of ELE-CuONPs. Further, the Fourier transformed infrared (FTIR) analysis of ELE-CuONPs also implicated other functional bio-molecules like proteins, sugars, alkenes, etc. with ELE terpenoids corona. Flow cytometric (FCM) data exhibited significantly enhanced intracellular uptake propensity of terpenoids encapsulated ELE-CuONPs and accumulation of intracellular reactive oxygen species (ROS), which ensued killing of planktonic cells of extended spectrum β-lactamases (ESβL) producing *Escherichia coli*-336 (E. coli-336), *Pseudomonas aeruginosa*-621 (P. aeruginosa-621) and methicillin-resistant *Staphylococcus aureus*-1 (MRSA-1) clinical isolates compared to the bare surface commercial nano-CuO and bulk sized CuO. The study for the first-time demonstrated the (i) differential bio-nano interface activities due to ELE surface and varied cell wall composition of test bacterial isolates, (ii) antibacterial effect and biofilm inhibition due to disruption of proteins involved in adhesion and biofilm formation triggered by CuONPs induced intracellular oxidative stress, and (iii) indigenous terpenoids-capped bio-inspired CuONPs are more stable and effective antibacterial and antifilm agent as compared with commercially available nano-CuO and bulk-CuO.

1. Introduction

Development of nanomaterials using natural bio-resources as eco-friendly, safe, inexpensive, and viable alternative to chemical-based synthesis is gaining much credence in manufacturing nanomaterials. Largely, the chemical methods result in adsorption of toxic chemicals on particle surface, which may cause adverse health effects. Therefore, biogenic/bio-inspired approach using plants such as neem [1], alfalfa [2–3], *Cinnamomum camphora* [4], *Emblica officinalis* [5], lemon grass [6], tamarind [7] and *Euphorbia tirucalli* [8] has been regarded as a green route for nanoparticles (NPs) synthesis.

The unique properties of semi-conductive transitional metal oxides are specifically dedicated to electronics, solar energy transformation, gas sensors and catalysts industries [9–12]. Amongst metal oxides, the copper oxide (CuO) and cupric oxide (Cu₂O) are known as p-type semiconductors [13], with a narrow band gap and as a powerful...
In vitro investigation to explore the toxicity of different groups of pesticides for an agronomically important rhizosphere isolate *Azotobacter vinelandii*

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**ABSTRACT**

In this work, an attempt was made to evaluate the effect of pesticides on growth pattern, surface morphology, cell viability and growth regulators of nitrogen fixing soil bacterium. Pesticide tolerant *Azotobacter vinelandii* strain AZ6 (Accession no. MG028654) was found to tolerate maximum level of pesticide and displayed multifarious PGP activities. At higher concentrations, pesticides triggered cellular/structural damage and reduced the cell viability as clearly shown under SEM and CLSM. With increase in concentration, pesticides exhibited a significant (p < 0.05) decrease in PGP traits of strain AZ6. Among all three groups of pesticides, herbicides glyphosate and atrazine were most toxic. Kitazin, hexaconazole, metalaxyl, glyphosate, quizalofop, atrazine, fipronil, monocrotophos and imidacloprid at 2400, 1800, 1500, 900, 1200, 900, 1800, 2100 and 2700 μg L\(^{-1}\), respectively, decreased the production of IAA by 19.5 ± 1.9 (61%), 18.1 ± 1.2 (64%), 36.4 ± 3.4 (28%), 13.1 ± 0.8 (74%), 15.6 ± 1.0 (69%), 7.6 ± 0.5 (83%), 11.9 ± 0.8 (76%), 24.7 ± 1.7 (51%) and 32 ± 2.3 (37%) μg mL\(^{-1}\), respectively, over control (50.7 ± 3.6 μg mL\(^{-1}\)). A maximum reduction of 8.4 ± 1.2 (46%), 5.8 ± 0.6 (62%) and 4 ± 0.2 (74%) μg mL\(^{-1}\) in 2, 3-DHBA at 300 (1×), 600 (2×) and 900 (3×) μg mL\(^{-1}\) respectively, With 32.8 ± 2.7 (19%), 27.2 ± 2 (33%) and 21.5 ± 1.3 (47%) μg mL\(^{-1}\), respectively in the production of SA was observed at 300 (1×), 600 (2×) and 900 (3×) μg mL\(^{-1}\) atrazine, respectively. Likewise, with increase in concentration of pesticides, decrease in P solubilization ability and change in pH of broth was detected. The order of pesticide toxicity to PSE (percent decline over control) at highest concentration was: atrazine (45%) > kitazin (44%) > metalaxyl (43%) > monocrotophos (43%) > glyphosate (41%) > hexaconazole (39%) > quizalofop (33%) > imidacloprid (31%) > fipronil (25). The present study undoubtedly suggests that even at higher doses of pesticides, *A. vinelandii* maintained secreting plant growth regulators and this property makes this strain agronomically important microbe for enhancing the growth of plants.

**1. Introduction**

In modern era, rapid industrialization has led to the production of various agrochemicals, synthentic pesticides, fertilizers and other chemicals. Among them, pesticides are new age miracle to agricultural industry, due to the better yield and reduced labour expenses by eradicating the pests that would else harmful to the crops (Simmons, 2017). Herbicides kill unwanted weeds (Sherman and Vaughn, 2018), insecticides the insects that would otherwise feed on crops (Sarwar, 2015) and fungicides prevent numerous diseases caused due to fungal pathogens (Roux et al., 2019). These chemicals when indiscriminately and judiciously applied in agricultural practices, a major fraction of these plant protection agents is accumulated in soil systems and adversely affect soil fertility (Maddela and Venkateswarlu, 2018), microbial diversity (Sun et al., 2017), metabolic activities of micro-organisms (Wu et al., 2018), soil biochemical processes such as nitrogen fixation (Ju et al., 2017), nitrification, ammonification and P solubilization.

As pesticides are used in agricultural practices to control the harmful pests that are responsible for decreased crop productivity. Some pesticides used to protect the crops, can be harmful to *Azotobacter* and it may cause deleterious impacts. In this context, Askar and Kudhur (Askar and Kudhur, 2013), have been reported that recommended doses of imazetapir, dimethoate and bayleton negatively effects the growth, viability, nitrogen fixation and nif genes of *A. chroococcum* and *A. vinelandii*. Plant growth regulators like IAA and P-solubilization of some soil bacteria are negatively affected by some synthetic pesticides (Madhaiyan et al., 2006). In a similar study, insecticide chlorpyrifos...
Fungicide tolerant *Bradyrhizobium japonicum* mitigate toxicity and enhance greengram production under hexaconazole stress

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**ARTICLE INFO**

**ABSTRACT**

Bacterial strain RV9 recovered from greengram nodules tolerated 2400 μg/mL of hexaconazole and was identified by 16S rDNA sequence analysis as *Bradyrhizobium japonicum* (KY940048). Strain RV9 produced IAA (61.6 μg/mL), ACC deaminase (51.7 mg/(protein·hr)), solubilized TCP (105 μg/mL), secreted 337.6 μg/mL EPS, and produced SA (52.2 μg/mL) and 2,3-DHBA (28.3 μg/mL). Exopolysaccharides produced by strain RV9 was quantified and characterized by SEM, AFM, EDX and FTIR. Beyond tolerance limit, hexaconazole caused cellular impairment and reduced the viability of strain RV9 revealed by SEM and CLSM. Hexaconazole distorted the root tips and altered nodule structure leading thereby to reduction in the performance of greengram. Also, the level of antioxidant enzymes, proline, TBARS, ROS and cell death was increased in hexaconazole treated plants. CLSM images revealed a concentration dependent increase in the characteristic green and blue fluorescence of hexaconazole treated roots. The application of *B. japonicum* strain RV9 alleviated the fungicide toxicity and improved the measured plant characteristics. Also, rhizobial cells were localized inside tissues as revealed by CLSM. Colonization of *B. japonicum* strain RV9 decreased the levels of CAT, POD, APX, GPX and TBARS by 80, 5, 13, 13 and 19%, respectively over plants grown at 80 μg/(hexaconazole·kg soil). The ability to detoxify hexaconazole, colonize plant tissues, secrete PGP bioactive molecules even under fungicide pressure and its unique ability to diminish oxidative stress make *B. japonicum* an attractive choice for remediation of fungicide polluted soils and to concurrently enhance greengram production under stressed environment.

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**Keywords:** Hexaconazole toxicity, Greengram, *Bradyrhizobium japonicum*, Cellular damage, Nodule ultrastructure, Oxidative stress

**Introduction**

Fungicides are repeatedly and harshly used in various cropping systems including those of legumes to control several soil-borne diseases. Due to extensive and injudicious use, major portion of the fungicides applied in superfluous quantity persists in soils (Gamiz et al., 2016). The accumulation of fungicides within soils and its uptake later on by plants, cause toxicity to legumes (Mohamed and Akladious, 2017). Greengram (*Vigna radiata* (L.) Wilczek) among legumes is an important pulse crop which provides high protein (20%–25%) and carbohydrate (60%–65%) in human foods (Swain et al., 2014). Although greengram is cultivated over a large area, it suffers heavily from several biotic and abiotic factors which limit its production (Deepa et al., 2017). Greengram attacked by several pathogens results in extensive yield losses (Tripathy, 2017) which could be up to 44% by fungal pathogens alone (Iqbal et al., 2014). However, to eliminate the fungal...
Understanding the phyto-interaction of heavy metal oxide bulk and nanoparticles: evaluation of seed germination, growth, bioaccumulation, and metallothionein production

Bilal Ahmed, Asfa Rizvi, Almas Zaidi, Mohammad Saghir Khan and Javed Musarrat

The fast-growing use of nano-based products without proper care has led to a major public health concern. Nanomaterials contaminating the environment pose serious threat to the productivity of plants and via food chain to human health. Realizing these, four vegetable crops, radish, cucumber, tomato, and alfalfa, were exposed to varying concentrations of heavy metal oxide (TiO₂, ZnO, Al₂O₃ and CuO) submicron or bulk (BPs) and nanoparticles (NPs) to assess their impact on relative seed germination (RSG), seed surface adsorption, root/shoot tolerance index (RTI/STI), bioaccumulation, and metallothioneins (MTs) production. The results revealed a clear inhibition of RSG, RTI, and STI, which, however, varied between species of metal-specific nanoparticles and plants. SEM and EDX analyses showed significant adsorption of MONP agglomerates on seed surfaces. The concentration of metals detected by EDX differed among vegetables. Among the metals, Al, Cu, Ti, and Zn were found maximum in alfalfa (12.46%), tomato (23.2%), cucumber (6.32%) and radish (21.74%). Of the four metal oxides, ZnO was found most inhibitory to all vegetables and was followed by CuO. The absorption/accumulation of undesirable levels of MONPs in seeds and seedlings differed with variation in dose rates, and was found to be maximum (1748–2254 μg g⁻¹ dry weight) in ZnO-NPs application. Among MONPs, the uptake of TiO₂ was minimum (2 to 140 μg g⁻¹) in radish seedlings. The concentration of MTs induced by ZnO-NPs, ZnO-BPs, and CuO-NPs ranged between 52 and 136 μmol MTs g⁻¹ FW in vegetal organs. Conclusively, the present findings indicated that both the nanosize and chemical composition of MONPs are equally dangerous for vegetable production. Hence, the accumulation of MONPs, specifically ZnO and CuO, in edible plant organs in reasonable amounts poses a potential environmental risk which, however, requires urgent attention to circumvent such toxic problems.

1. Introduction

Nano-technological advancements on the one hand have great potential in many environmental and industrial applications, while on the other hand they raise serious concerns over the use of NPs due to environmental problems. Among various NPs, metal oxide nanoparticles (MONPs) for example, ZnO, CuO, TiO₂, Al₂O₃, ZrO₂, Fe₂O₃, Ag₂O, CeO₂, and NiO are widely used in many industries such as cosmetics, energy production, paints, textiles, and rocket fuels, and in biomedical applications. Apart from these, MONPs have also been applied in agriculture practices as nano-fertilizers and in protecting plants from pathogens.

Due to the ever-increasing demands, it is likely that the production of MONPs which was just 0.27 million tons in 2012 will increase to 1.663 million tons by 2020. Of the total production, 8–28%, 0.4–7.0%, and 0.1–1.5% MONPs are expected to accumulate in the soil system, water and atmosphere, respectively, after production, application, and discharge. Once deposited in soil either through nano-products such as fertilizers, insecticide, and pesticides or from other sources, the MONPs may become toxic to bacteria, plants, animal, and human cells. Despite these, the understanding on lethality of MONPs is still limited and hence, requires special attention to better understand the consequences of MONPs on crop production. However, in this context, a very few attempts have been made to assess the biological impacts of NPs in controlled laboratory conditions with single species of model organisms, which are essential to elucidate the interaction mechanism of NPs.

Indeed, plants are critical for the sustenance of the ecosystem, and due to the direct association of roots with soil ecosystem, plants come in direct contact with soil constituents...
Bioreduction of toxicity influenced by bioactive molecules secreted under metal stress by *Azotobacter chroococcum*

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Abstract
Heavy metal pollution destruct soil microbial compositions and functions, plant’s performance and subsequently human health. Culturable microbes among many metal abatement strategies are considered inexpensive, viable and environmentally safe. In this study, nitrogen fixing bacterial strain CAZ3 recovered from chilli rhizosphere tolerated 100, 1000 and 1200 µg mL⁻¹ of cadmium, chromium and nickel, respectively and was identified as *Azotobacter chroococcum* by 16S rDNA sequence analysis. Under metal stress, cellular morphology of *A. chroococcum* observed under SEM was found distorted and shrinkage of cells was noticed when grown with 50 µg mL⁻¹ of Cd (cell size 1.7 µm) and 100 of µg mL⁻¹ Ni (cell size 1.3 µm) compared to untreated control (cell size 1.8 µm). In the presence of 100 µg mL⁻¹ of Cr, cells became elongated and measured 1.9 µm in size. Location of metals inside the cells was revealed by EDX. A dose dependent growth arrest and consequently the death of *A. chroococcum* cells was revealed under CLSM. *A. chroococcum* CAZ3 secreted 320, 353 and 133 µg EPS mL⁻¹ when grown with 100 µg mL⁻¹ each of Cd, Cr and Ni, respectively. The EDX revealed the presence of 0.4, 0.07 and 0.24% of Cd, Cr and Ni, respectively within EPS extracted from metal treated cells. Moreover, a dark brown pigment (melanin) secreted by *A. chroococcum* cells under metal pressure displayed tremendous metal chelating activity. The EDX spectra of melanin extracted from metal treated cells of *A. chroococcum* CAZ3 displayed 0.53, 0.22 and 0.12% accumulation of Cd, Cr and Ni, respectively. The FT-IR spectra of EPS and melanin demonstrated stretching vibrations and variations in surface functional groups of bacterial cells. The C-H stretching of CH₃ in fatty acids and CH₂ groups, stretching of N-H bond of proteins and O-H bond of hydroxyl groups caused the shifting of peaks in the EPS spectra. Similar stretching vibrations were recorded in metal treated melanin which involved CHO, alkyl, carboxylate and alkene groups resulting in significant peak shifts. Nuclear magnetic resonance (NMR) spectrum of EPS extracted from *A. chroococcum* CAZ3 revealed apparent peak signals at 4.717, 9.497, 9.369 and 9.242 ppm. However, ¹H NMR peaks were poorly resolved due largely to the impurity/viscosity of the EPS. The entrapment of metals by EPS and melanin was confirmed by EDX. Also, the induction and excretion of variable amounts of metallothioneins (MTs) by *A. chroococcum* under metal pressure was interesting. Conclusively, the present findings establish- (i) cellular damage due to Cd, Cr and Ni and (ii) role of EPS, melanin and MTs in adsorption/complexation and concurrently the removal of heavy metals. Considering these, *A. chroococcum* can be promoted as a promising candidate for supplying N efficiently to plants and protecting plants from metal toxicity while growing under metal stressed environment.

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Putative Role of Bacterial Biosorbent in Metal Sequestration Revealed by SEM–EDX and FTIR

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Abstract Bacterial exopolysaccharides (EPS) play a critical role in sequestration of metals from contaminated environment. Considering these, this study was aimed at extracting EPS from metal tolerant Pseudomonas aeruginosa CPSB1 and Azotobacter chroococcum CAZ3 and to ascertain its role in metal removal. P. aeruginosa CPSB1 and A. chroococcum CAZ3 secreted 1306.7 and 1660 μg mL⁻¹ EPS, respectively in the presence of 200 and 100 μg mL⁻¹ Pb, respectively with glucose as C source. The binding of metal ions to bacterial EPS was validated by SEM and EDX. The functional group involved in metal chelation was revealed by FT-IR. The metal ions were adsorbed onto EPS and hence, EPS could play a crucial role in metal detoxification. Due to this novel trait, P. aeruginosa CPSB1 and A. chroococcum CAZ3 could be developed as bioinoculant to cleanup metal contaminated sites.

Keywords Exopolysaccharides · Heavy metals · Metal sequestration · P. aeruginosa · A. chroococcum

Heavy metals are significant environmental threat, which, when present beyond threshold levels in the environment, disrupt the ecological niches. However, certain metal tolerant bacterial strains have been found potentially magical in detoxifying polluted environment. For this, the metal tolerant bacterial strains have evolved multiple strategies to remediate metal contaminated soils. For example, metal biosorption, extracellular precipitation, conversion of toxic metal ions into less toxic forms and flush out (efflux pumping) of metals to exterior environment are some of the approaches adopted by bacteria to thrive well even under metal stressed conditions [1]. Apart from these, bacterial cells also synthesize extracellular polymeric substances which allow them to survive even in the presence of stressor molecules by masking their toxic impact [2, 3]. Also, EPS plays a significant role in metal chelation, wherein, the ionic forms of metals bind to the complex polymeric structures of EPS. The secretion of EPS by bacterial cells is thus an interesting biological phenomenon to shield themselves from the harsh environment by forming a biofilm matrix on solid substrates. Moreover, the structure and composition of EPS is such that it amply allows easy sequestration of metal ions. Due to these functional properties, microbial polymers have largely been employed in metal removal from polluted environments [4]. Considering the importance of EPS in metal sequestration and detoxification, the present study was aimed at searching metal tolerant bacterial strains capable of synthesizing EPS under both metal stressed and conventional environments. Also, the uptake and localization of metals within EPS was determined by SEM and EDX while functional moieties of EPS were detected by FTIR analysis.

The production of EPS by metal tolerant bacterial strains under conventional and metal stressed conditions was determined by growing P. aeruginosa CPSB1 and A. chroococcum CAZ3 in 50 mL nutrient broth containing 5% glucose and treated with 0, 25, 50, 100 and 200 μg mL⁻¹ each of Cu, Cd, Cr, Ni and Pb. The bacterized flasks were incubated at 28 ± 2 °C for 5 days on a rotary shaker incubator at 120 r min⁻¹. The culture broth was spun at 8000 rpm for 30 min and three volume of chilled acetone

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Heavy metal mediated phytotoxic impact on winter wheat: oxidative stress and microbial management of toxicity by Bacillus subtilis BM2

Asfa Rizvi, Bilal Ahmed, Almas Zaidi and Mohd. Saghir Khan

Heavy metals are toxic environmental contaminants, which severely affect microbial composition and functions and, concurrently, crop production. Due to these issues, the present study focussed on the selection of metal tolerant microbes endowed with metal detoxification abilities and their role in the management and remediation of metal contaminated soils. The metal tolerant bacterium BM2, identified as Bacillus subtilis by 16S rRNA gene sequencing, survived well under metal pressure and tolerated 1600 and 2000 μg mL⁻¹ of Ni and Pb, respectively. The inhibitory impact of metals on wheat increased consistently with a progressive increase in metal concentration. Deposition of Ni and Pb within root and leaf and oxidative stress were validated by SEM, EDX and CLSM. The overall growth parameters of wheat grown under metal stress were improved following B. subtilis BM2 colonization. As an example, B. subtilis with 195 mg Pb kg⁻¹ enhanced the length and dry biomass of shoots by 14% and 23%, respectively, over the control. Also, strain BM2 improved the grain yield significantly by 49% at 870 mg Ni kg⁻¹ and by 50% at 585 mg Pb kg⁻¹ compared to uninoculated plants. Moreover, B. subtilis BM2 relieved the metal stress on wheat and caused a significant drop in proline and malondialdehyde content and the activities of antioxidant enzymes, like catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR). This study, therefore, provided solutions to the metal toxicity problems faced by winter wheat and clearly suggests that the metal detoxification potential of B. subtilis BM2 could be greatly useful in the management of metal polluted soils.

1. Introduction

Among various soil pollutants, heavy metal pollution is one of the major global challenges and has attracted the attention of scientists to protect/preserve the very sustainability of natural ecosystems. In many developed and developing countries the heavy metal pollution has risen due to rapid industrialization, long term use of poor quality waters (untreated waste water) for irrigation and intensive agricultural practices. These anthropogenic activities in isolation or simultaneously add considerable amounts of heavy metals to soils. Unfortunately, heavy metals are biologically non-destructible constituents, and hence, they persist indefinitely in soil ecosystems. Once accumulated in soils, heavy metals adversely affect the soil dynamics and microbial composition and functions, leading eventually to losses in soil fertility and ultimately crop production. From plants, metals can be introduced into the food chain, which subsequently raises the risk of toxicity to both animals and humans. However, not all metals are inhibitory at any given concentration, but rather their toxicity and deleterious impact differ from species to species and with the concentrations of metals as well as the age and genotypes of plants. Indeed, some metals are essential and play important roles in regulating various metabolic functions of plants. For example, while high concentrations of Ni negatively affect plant growth, as a trace micronutrient element, it aids in regulating physiology including seed germination and nitrogen metabolism of plants. Some common symptoms associated with Ni phytotoxicity include (i) leaf chlorosis, (ii) abnormal root/shoot growth, (iii) deformity in various plant organs and (iv) altered nutrient uptake. Due to these effects, enhanced accumulation of Ni inside plant tissues results in massive losses in both quantity and nutritive quality of agronomically important and edible crops. Lead is another bioactive metal which, at elevated levels, decreases cell division in plants and causes brittleness in leaves with the appearance of dark purple spots. Imbalance in water transport, disturbed composition and uptake of mineral nutrients, interruption of photosynthesis, altered enzymatic activities and overall hormonal imbalance are a few other significant symptoms of Pb toxicity in plants. Apart from the direct inhibitory effect of metals on growing plants, metals can also destroy cells by causing overproduction of reactive oxygen species (ROS), which damage/impair antioxidant defense systems resulting in oxidative stress.
Chromium-reducing and phosphate-solubilizing Achromobacter xylosoxidans bacteria from the heavy metal-contaminated soil of the Brass city, Moradabad, India

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Abstract
Chromium contamination in soil and water bodies is increasing predominantly due to inappropriate discharge from industries, and it is causing severe environmental problems and soil infertility. To improve soil quality, the sustainable approach needs to identify specific microbes capable of reducing chromium toxicity, enhancing soil P pool and expressing multiple plant growth-promoting activities. In the current investigation, a microbial strain OS2 was recuperated from polluted soil and was characterized by employing biochemical and molecular methods. Bacterial strain OS2 was identified as Achromobacter xylosoxidans by 16S rRNA quality sequencing, BLASTn, and phylogenetic examination. Strain OS2 survived well at high doses of heavy metals: Cr, Ni, Cu and Zn. A. xylosoxidans could solubilize up to 363 µg mL⁻¹ tricalcium phosphate and reduced 100 µg mL⁻¹ chromium after 24-h incubation. SEM and EDX analyses showed the highest accumulation of phosphate and binding with chromium up to 10.22 and 1.09 weight percent of total weight, respectively. A. xylosoxidans significantly produced IAA (26 µg mL⁻¹) when grown up within 100 µg mL⁻¹ chromium, as detected by HPLC. Further, strain OS2, when used as a microbial inoculant, decontaminated the chromium and concurrently improved the growth of mung bean plants while growing under metal stress conditions significantly in a sustainable manner.

Keywords Chromium reduction · Phosphate solubilization · Achromobacter xylosoxidans · Siderophores · IAA · Heavy metal

Introduction
Worldwide modernization is raising the expansion of industrialization, urbanization, pollution and global warming (Kingsley et al. 1962). Numerous anthropogenic activities and various industries such as mining, metallurgy, electroplating, tanneries, paint, cement, ceramic and fertilizer industries are releasing a huge amount of wastes which contain the alarmingly high level of toxic metals (McDonagh et al. 2012; Lyu et al. 2016; John et al. 2016). Soil biologists in recent times have reported about the worrying situation of heavy metal pollution in our surroundings, which are unpleasantly affecting ecosystems and biodiversity (Wang et al. 2007; Chen et al. 2014). Massive amounts of heavy metals discharged from various sources are continually entering into food chains and consequently severely affecting the metabolism, leading eventually to the death of microbes, plants and animals (Theriault and Nkongolo 2016; Fernando et al. 2016). For example, Cd, Cr, Pb, Hg, Ni and Zn are the most toxic elements, even at low concentration, and have been found to disrupt the metabolism of organisms and also to cause human health problems (Eroğlu et al. 2016; Zhang et al. 2016). Hexavalent chromium among metals is a toxic contaminant which arises in the environments from metal furnishing and electroplating industries, cooling tower, tanning, and dyes and paints processing industries. Interestingly, chromium occurs in two to six different oxidation states. Of these, the most toxic and carcinogenic
Impact of toxic metals on microbially synthesized plant active biomolecules and metal tolerant *Bradyrhizobium* inoculated Mungbean [*Vigna radiata* (L.) Wilczek]

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Abstract

The rhizobacterial strains recovered from different rhizospheres were characterized and exposed to potassium dichromate and chloride in order to discover metal tolerant bacterial strains with multiple plant growth promoting activities. A total of four metal bacterial strains belonging to genera *Pseudomonas*, *Bradyrhizobium*, *Azorobacter*, and *Bacillus* were selected and evaluated for plant growth promoting potential under heavy metal stress. All strains were though positive for ammonia and siderophore production, but *B* showed poor ammonia production at higher concentration of Cr (VI). Furthermore, the metal tolerant and plant growth promotive *Bradyrhizobium* sp. augmented the growth of mungbean grown with varying rates of heavy metals. The present study suggest toxic metals indeed had inhibitory impact on survivability of potential microflora but they did not completely abolish their physioactivities even under metal horror conditions. Due to these, the metal tolerant PGPR could be useful as inoculant for enhancing overall performance of mungbean when grown even under metal enriched soils.

Keywords

Heavy metals, Metal tolerant PGPR, Plant biomolecules, *Vigna radiata*.
Effective Inhibition of Phytopathogenic Microbes by Eco-Friendly Leaf Extract Mediated Silver Nanoparticles (AgNPs)

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Abstract This study was aimed at producing the eco-friendly, safe, and inexpensive silver (Ag) nanoparticles (NPs) and assessing its antimicrobial activity. Fungal pathogens isolated from diseased leaves and fruits of brinjal and bacterial pathogen obtained from a culture collection were used in this study. Green synthesis of AgNPs was performed and optimized using Azadirachta indica leaf extract. The newly synthesized AgNPs (λmax = 437 nm) showed isotropism in size (crystal size/diameter: 21/29 ± 5 nm) and morphology under transmission and scanning electron microscopy and energy dispersive X-ray analysis. The fourier transform infrared spectroscopy data suggested the role of various aliphatic/aromatic moieties and proteins in AgNPs stabilization. The AgNPs reduced the growth of Penicillium sp. maximally by 92% after 6 days. The sensitivity of test fungi towards AgNPs followed the order: Penicillium sp. (92%) > Fusarium sp. (89%) > Aspergillus sp. (69%). Exposure of Ralstonia solanacearum to AgNPs (MIC/MBC 200/400 μg ml⁻¹) displayed damaged cellular envelopes, bulging of cells, and pit formation. The nucleic acid discharge showed a progressive increase from 8 to 34% (r² = 0.97). The cellular metabolic activity and surface adhering ability of R. solanacearum were completely lost at 400 μgAgNPs ml⁻¹. Results suggested that the AgNPs synthesized in this study had enough anti-pathogenic potential and could inexpensively and safely be used as a promising alternative to agrochemicals. Moreover, the findings observed in this study is likely to serve as an important indicator for the development of effective nano-control agents which in effect would help to manage some deadly phyto-pathogens capable of causing heavy losses to agricultural production systems.

Graphical Abstract Effective inhibition of phytopathogenic microbes by eco-friendly neem leaf extract mediated silver nanoparticles (AgNPs)

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Survival of probiotic bacteria in the presence of food grade nanoparticles from chocolates: an in vitro and in vivo study

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Abstract
The use of probiotics to treat gastrointestinal diseases such as diarrhea especially in children is becoming increasingly popular. Besides, the use of nanomaterials in food products is increasing rapidly especially in candies and chocolates. How these nanomaterials influence probiotic bacteria and their activity remains unexplored. Therefore, nanomaterials from commercial chocolate were purified and characterized by using SEM–EDS and XRD. The tested chocolate contained nano-TiO 2 with an average size of ~ 40 nm. The influence of the extracted TiO 2 on a commercial probiotic formulation usually used to treat diarrhea in children was studied. The probiotic formulation contained Bacillus coagulans, Enterococcus faecalis, and Enterococcus faecium as evident from 16S rRNA gene sequences and polyphasic characterization. Isolated bacteria exhibited known probiotic activities like biofilm formation, acid production, growth at 6% salt, and antibiotic resistance. TiO 2 from chocolates inhibited the growth and activity of the probiotic formulation over a concentration range of 125–500 μg/ml in vitro. Based on results, it is estimated that 20 g of such chocolate contains enough TiO 2 to disturb the gut microbial community of children aged 2–8 years with a stomach capacity of ~ 0.5–0.9 l. The in vivo study on white albino mice shows the same response but with a higher dose. The results obtained by plate counts, MTT assay, live/dead staining, and qPCR suggest that TiO 2 from chocolates inhibits the growth and viability of probiotic bacteria in mice gut even at a concentration of 50–100 μg/day/mice. Therefore, TiO 2 in chocolate discourages survival of probiotic bacteria in the human gut.

Keywords Nano-TiO 2 · Nano-silver · Chocolate, Probiotics

Introduction
The use of probiotic bacteria for human health has been practiced since ancient times (McFarland 2015). However, recently, their use has increased enormously as apparent from the rise of probiotic food products and supplements in the market (Hajela 2014; Reid 2015). The recent commercial success of probiotic bacteria is known globally through probiotic based products like Yakult. The increasing knowledge about the significance of microbiome in human health may also have contributed to this renewed interest in probiotics. The link between the gut microbiome and human health is becoming increasingly clear and is well described (Clemente et al. 2012). Nonetheless, the gut microbiome continuously changes under the influence of a number of factors like diet, lifestyle, and consumption of antibiotic (Spor et al. 2011; Zuo et al. 2018). The use of probiotic supplements not only helps in the maintenance of a healthy gut microbiome but also improves the overall all health of humans (Fuller 1991; Valdes et al. 2018). Furthermore, probiotic microorganisms are now represented by more phylogenetically diverse microorganisms than previously thought and are not anymore limited to a few conventionally used bacteria (Holzapfel et al. 2001; Macfarlane and Cummings 1999).

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Bacterial Community Structure in Anaerobic Digesters of a Full Scale Municipal Wastewater Treatment Plant – Case Study of Dubai, United Arab Emirates

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ABSTRACT

A highly complex microbial community involved in anaerobic sludge digesters plays vital roles in sludge treatment. The data on microbial ecology is important to accomplish efficient operation of the anaerobic digesters. This study is aimed at monitoring the bacterial community of three full-scale anaerobic digesters of a full-scale municipal wastewater treatment Plant in Dubai, United Arab Emirates. Fluorescent in-situ hybridization technique was applied to identify the bacterial groups and quantitative polymerase chain reaction to compare the richness of bacterial and archaeal domain. Results of the fluorescent in-situ hybridization technique analysis showed that the phylum Proteobacteria was most abundant followed by cytophage-Flavobacterium group of Bacteroides, Firmicutes and Actinobacteria. Among proteobacterial subclass Delta- and Alpha- were dominating than Gamma- and Beta-proteobacteria. The genus Desulfobacter and Desulfo bacterium were the dominant groups hybridizing 70-76% of total 4', 6'-diamidino – 2 phenylindole stained cells. The quantitative polymerase chain reaction results showed that Bacterial domain was dominating in all three digesters compared to the archaeal domain.

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Chemical diversity in leaf and stem essential oils of *Origanum vulgare* L. and their effects on microbicidal activities

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Abstract

Essential oils (EOs) from the stems and leaves of *Origanum vulgare* L. grown in Saudi Arabia and Jordan were analyzed by gas chromatography–mass spectrometry (GC–MS) and GC–flame ionization detector (FID) techniques on two different columns (polar and nonpolar). A detailed phytochemical analysis led to the identification of 153 constituents of these essential oils. Both Saudi and Jordanian plants are classified by chemotypes rich in cymyl-compounds. However, the Saudi *Origanum* contains carvacrol as the major component and is, thus, characterized as a carvacrol chemotype, while the Jordanian *Origanum* contains thymol as the major component, and, thus, it is classified as a thymol chemotype. In addition, the antimicrobial activities of the studied EOs and their major components, including carvacrol and thymol, were evaluated against various Gram-positive and Gram-negative microorganisms. All the tested compounds exhibited significant antimicrobial activity against all the tested bacteria. Among them, thymol demonstrated superior activity against all the tested organisms, followed by carvacrol. Moreover, results on oil composition and oil yield of *O. vulgare* L. from different parts of the world is compared in detail with the present outcomes.

Keywords: Essential oils, NMR, GC–MS, *Origanum vulgare* L., Lamiaceae

Introduction

Recently, the demand for the development of natural products from medicinal and aromatic plants as substitutes for artificial additives and as pharmacologically active agents has increased significantly (Atanasov et al. 2015). Among the different natural products, essential oils (EOs) have gained immense popularity in various industries, including the food, cosmetics, and pharmaceutical industries, because of their remarkable characteristics such as, strong odor, unique colors, and high volatility (Carvalho et al. 2016; Maggio et al. 2016). In particular, EOs play a significant role in the health care sector by virtue of their remarkable biological activities, which are directly associated with their biologically active essential oil components (Raut and Karuppayil 2014).

EOs are oily substances produced by different parts of the plants, including flowers, buds, leaves, twigs, stems, seeds, and fruits (Bakkali et al. 2008). Generally, these oils are comprised of complex mixtures of volatile substances that are biosynthesized by plants. These substances can be broadly classified into several groups, such as aromatic and aliphatic compounds, terpenes, and terpenoids (Pichersky et al. 2006).

Most of the biological activities of EOs, particularly their antimicrobial activity, is associated with oxygenated terpenes, such as alcohols and phenolic terpenes. However, a few hydrocarbons have been found to exhibit significant antibacterial effects (Bassolé and Juliani 2012). Usually, the complex interactions between the diverse classes of phytoconstituents, such as phenols, alcohols, aldehydes, ketones, or other hydrocarbons of EOs are responsible for their antibacterial activities. In some cases, these interactions may lead to antagonistic or synergistic effects that contribute to the antibacterial activity of EOs, and even minor components of EOs can play...
Heavy metal-mediated toxicity to maize: oxidative damage, antioxidant defence response and metal distribution in plant organs

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Abstract
Heavy metals are serious environmental threats that, after accumulation inside edible organs of the food crops and following consumption, pose major human health problems. Maize is one of the most important food grain crops and so is the changes caused by heavy metals. Realizing this, the toxic impact of cadmium, chromium and nickel on biological characteristics, seed attributes, antioxidant enzymes and metal distribution in maize was assessed. Growth and yields of maize plants declined regularly with progressively increasing concentrations of metals. Among all metals, cadmium had most lethal effects and the onset of vegetative and reproductive growth stages was delayed. Cadmium at 36 mg/kg maximally declined the root and shoot length by 65 and 32%, respectively, while chromium (204 mg/kg) reduced the total chlorophyll content, grain yield and grain protein by 77, 84 and 16%, respectively, over control. The severity of oxidative stress increased with increasing rates of metals. Proline and malondialdehyde were enhanced by 59 and 72%, respectively, over control. The expression of antioxidant enzymes was superior in foliage of maize grown under metal stress. Cadmium maximally reduced the total P content in roots and shoots. Roots in general had more metals than shoots and grains. The scanning electron microscopy and confocal laser scanning microscopy images showed metal distorting impact while energy-dispersive X-ray spectroscopy confirmed the location of metals inside plant organs. The results suggest alarming consequences of metal toxicity to maize and the accumulation of heavy metals within grains raises disturbing public health concerns.

Keywords Abiotic stress · Cell surface damage · Confocal laser scanning microscopy · Maize · Phytotoxicity · Scanning electron microscopy

Introduction
Maize (Zea mays L.), cultivated globally, is the third most important cereal crop after wheat and rice (Akongwubel et al. 2012). Maize is cultivated in temperate and subtropical regions and can survive even under extreme environmental conditions (Maiti et al. 2012). Also, maize plant proficiently extracts heavy metals from metal-polluted soils (Aliyu and Adamu 2014). Thus, even though maize can serve as a potential candidate for phytoextraction, yet it is also adversely affected by heavy metals when grown under metal stress. Among various toxic metals, cadmium is highly dangerous to many plants including maize (Hussain et al. 2013; Xu et al. 2014) and results in stunted growth, alters membrane permeability leading eventually to the production of ROS (reactive oxygen species) (Ibrahim et al. 2017). The accumulation of ROS within plant tissues in turn causes the leakage of electrolytes and disrupts the integrity of cell membrane (Emamverdian et al. 2015). As a result, the oxidation of membrane proteins and lipids is usually disrupted, which results in cell death (Ibrahim et al. 2015). Chlorotic and necrotic leaves, browning of roots and distortions in embryonic tissues are some of the other devastating toxicity symptoms of cadmium on maize plants (Ling et al. 2017). Chromium is another highly toxic metal which hampers growth and yield of plants (Parmar and Patel 2015) including maize (Anjum et al. 2016a, b). Some of the toxicity symptoms associated with chromium includes chlorosis of leaves, impaired growth of both roots and shoots and wilting (Kumar and Chopra 2015). Once chromium enters inside...
Research article

Screening of polycyclic aromatic hydrocarbon degrading bacterial isolates from oil refinery wastewater and detection of conjugative plasmids in polycyclic aromatic hydrocarbon tolerant and multi-metal resistant bacteria

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1. Introduction

Environmental pollution caused by xenobiotics has now become a major issue of concern. Industrialization is a critical factor for the development of the economy of a country. Most of the industrial activities generate huge amounts of gaseous, liquid, or solid hazardous wastes. During the process of refining crude oil, large volumes of fresh water is used by refineries (Shpiner et al., 2009) and generate huge amount of wastewater (Mustapha et al., 2015).

Oil refinery being an important industrial sector produce wastes that contains various chemicals in a significant concentrations including oil and greases, phenols (creosols and xylenols), sulphides, ammonia, suspended solids, cyanides, nitrogenous compounds, heavy metals, mono and polycyclic aromatic hydrocarbons (Hardik et al., 2010; Dhananjayan et al., 2012; Hara and Marin-Morales, 2017; Bahri et al., 2018).

The indigenous microbes which are present in wastewater and soil have been found to degrade refinery pollutants such as PAHs either aerobically or anaerobically (Dhaker and Jain, 2011; Jain et al., 2011; Zhao et al., 2017) using different enzymes like mono- and dioxygenases, laccase, and peroxidase etc. which involves the oxidation of PAH rings (Haritash and Kaushik, 2009). Gram negative bacterial community has been reported to be more efficient PAHs degraders (Ahmad et al., 2019).

Several bacteria including Acinetobacter calcoaceticus, Alcaligenes denitrificans, Alcaligenes odorans, Arthrobacter polychromogenes, Bacillus thuringiensis, Burkholderia cepacia, Mycobacterium vanbaalenii, Mycobacterium flavescens, Pseudomonas aeruginosa, Pseudomonas putida, Sphingomonas paucimobilis, Stenotrophomonas maltophilia etc have been reported to efficiently degrade the PAHs (Liu et al., 2017).

It is reported that the abundance of plasmids is more in polluted sites than unpolluted zone; however experimental data are limited (Smalla et al., 2006; Heuer et al., 2009; Dealtry et al., 2016). Genes coding for enzymes that enable bacteria to resist antibiotics or heavy metals or to

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2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Mutagenicity, genotoxicity and oxidative stress induced by pesticide industry wastewater using bacterial and plant bioassays

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\textbf{A B S T R A C T}

Atomic absorption spectrophotometer and gas chromatography analysis revealed the presence of heavy metals, organochlorine and organophosphate pesticides in industrial wastewater. XAD, Dichloromethane and n-Hexane extracted wastewater were analysed for genotoxic potential using Ames \textit{Salmonella} mammalian test. The XAD concentrated sample displayed remarkable mutagenic activity compared to solvent assisted liquid–liquid extraction. Strain TA98 was found utmost sensitive towards all extracts. Wastewater induced chromosomal aberrations in roots of \textit{Allium cepa} showed significant (p < 0.05) decrease in mitotic index. Seeds of \textit{Vigna radiata} germinated on soft agar plates treated with different concentration of wastewater showed significant reduction in germination (32 \%), seedling vigor index (76 \%), radicle length (56 \%), plumule length (47 \%), biomass of radicle (64 \%) and plumule (57\%) at highest wastewater concentration. Propidium iodide stained \textit{V. radiata} roots showed oxidative stress induced by wastewater under CLS microscopy. Further, genotoxicity of wastewater was confirmed by plasmid nicking assay using pBR322 plasmid.

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1. Introduction

One of the leading causes of water pollution is the unchecked release of wastewater from various industries into water bodies and many other environments \cite{1,2}. The generation of wastewater is mostly due to rapidly growing industrial sector \cite{3} for the development and expansion of the nation’s economy. Amongst the innumerable industries, the pesticide industry is counted as one of the key contributors of water contamination.

Organochlorine (OC) and organophosphorus (OP) pesticides are most important contaminants released by pesticide industry around the world as well as in India \cite{4,5}. The existence of pesticide residues in water and soils impact on the vegetables as well as fruits and thus poses grave danger to human health. Many findings displayed that even very low level of pesticides cause natal defects \cite{6}. Numerous scientific endeavours in the area of genotoxicity of wastewater suggested direct association with mutagenicity of pollutants into water bodies \cite{7,8}. Several industrial wastewater effluents and sludges has shown high mutagenic potential \cite{9,10}.

With the fast pace in the development and era of modern mechanization the problem of pollution, specifically water pollution has been increased alarmingly in numerous developing countries including India \cite{11–13}. A lot of toxicants in the environment act by damaging of DNA and therefore causing mutations \cite{14–16}. Genotoxicity evaluation of industrial effluents on surface water indicates the presence of mixtures comprise of various toxic substances that may stand risk of hazard and carcinogenicity \cite{17,18}.

Biological assays with prokaryotic system detect mutagenic agents that persuade the gene level mutation and primarily damages the DNA. In contrast, eukaryotic based bioassay revealed exposure of a more degree of injury/impairment, variable from gene mutations to chromosomal aberrations and aneuploidies \cite{19}. Applying both the prokaryotic and eukaryotic based detection systems reinforce and relate the observations to make certain if the substances actually hold any adverse effects on the genetic materials.

Ames \textit{Salmonella}/microsomal test is extensively applied in examining the mutagenic potential of toxic chemicals \cite{20,21}. \textit{A. cepa} plant model is also extensively used for the evaluation of genotoxicity due to high sensitivity towards the xenobiotic compounds \cite{22}. Mung bean (\textit{Vigna radiata}) seed is another important short-term assay for genotoxicity evaluation using different parameters such as seed germination, seedling vigour.
Mutagenicity and genotoxicity evaluation of textile industry wastewater using bacterial and plant bioassays

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ABSTRACT

Textile industrial wastewater samples were taken from the Panki site 5 industrial area of Kanpur city, India. Atomic Absorption spectrophotometer and Gas Chromatography-Mass spectrometry techniques have shown that the wastewater contained several heavy metals and organic pollutants (Khan and Malik, 2017) [1]. Further, in order to explore the potential toxicity of these pollutants present in the effluent, a battery of short-term biological assays (Ames test, DNA repair defective mutation assay and Allium cepa chromosomal aberration test) were used. Wastewater samples were concentrated with XAD-4/8 resins and liquid-liquid extraction procedure. XAD-concentrated samples were more mutagenic than the liquid-liquid extracted samples. Ames TA98 and polA (SOS defective) strains were the most responsive strains. The wastewater also resulted in significant decline in mitotic index and induced chromosomal aberrations in A. cepa roots. The findings thus showed that the combination of physico-chemical analysis along with the toxicity assessment (using short term biological assays) would provide valuable and more realistic information about the joint toxicity of chemical pollutants present in the textile effluent.

1. Introduction

In almost all developing and in many of the highly developed nations, water pollution due to discharge of inadequately treated wastewater into the environment is a major issue of concern [2]. The wastewater generation is mainly attributed to the rapid increase in industrial sector, which is growing substantially for economic development of a country. Among the various industries, textile industry is considered as one of the major contributors of the water pollution, since the waste generated is very complex in nature containing color content and toxic components [3]. Textile wastewater constitutes a large number of chemicals like acids, bases, salts, dispersants, etc. [4-6], and most importantly dyes that altogether are responsible for high biological oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) [7]. Dyes therefore are one of the most important constituents of the textile industry and are major polluters of water reservoirs [8].

The direct discharge of the effluent in rivers not only adversely affects flora and fauna but is the cause of various human illnesses. In spite of the harmful consequences, textile effluent is being continuously released into water streams without any prior treatment or sometimes after partial treatment which deteriorates the quality of receiving water. The increasing discharge of these hazardous chemicals into the environment severely affect the natural ecosystems [9], and have adverse effects on human and environmental health [10].

In order to minimize the environmental deterioration due to exposure of textile wastewater, the effluent should be monitored carefully using multidisciplinary approach that involves a combination of chemical and biological methods. The wastewater can be assessed by means of various chemical methods, which require the standardization of thousands of organic pollutants present in the environment, making them tedious and time consuming processes. Moreover, the methods are not sufficient to assess the joint toxicity of the pollutants present in a mixture at low concentrations. Therefore, biological assessment method is required in order to detect the combined effects of chemical pollutants present in a mixture in the environment [11]. Biological method of assessment employs both prokaryotic and eukaryotic systems and can detect DNA damage from point mutations to chromosomal alterations [12].

Among the prokaryotic system, Ames test is one of the most widely used, short-term mutagenicity bioassay involving specifically designed Salmonella typhimurium strains with different pre-existing mutations in the histidine operon, making them unable to synthesize histidine amino acid [13-17]. Any chemical substance that may cause mutations at or near the histidine operon restores the “his+” gene function and results in growth of the bacteria in the absence of histidine. The Ames strains can...
Bio-inspired nanomaterials in agriculture and food: Current status, foreseen applications and challenges

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ABSTRACT

Nanotechnology is a potential area that revolutionizes almost every sector of life and is predicted to become a major economic force in the near future. Recently, nanomaterials have received great attention for their properties at nanoscale regime and their applications in many areas primarily, agriculture and food sectors. The Nanomaterials are dispersed or solid particles, with a size range of 1–100 nm. In recent times, there has been an increased research work in this area to synthesize nanomaterials using various approaches. The use of natural biomolecules using ‘green’ approach play key role in the synthesis of nanomaterials having different shapes and sizes. Further this ‘green synthesis’ approach not only minimize the cost but also limit the need of hazardous chemicals and stimulates synthesis of greener, safe and environmentally friendly nanoparticles. The present review focus on studies based on the biosynthesis of nanoparticles using biomolecules such as plants, bacteria, fungi, etc. The text summarizes the recent work done globally by renowned researchers in area of biosynthesis of nanomaterials. It also discusses the potential applications of biologically mediated nanomaterials in the areas of agriculture and food and a critical evaluation of challenges within this field.

1. Introduction

Nanotechnology is a cutting-edge technology that deals with nanosized materials [1]. It is a multidomain field, which covers diverse domains from engineering, biology, physics and chemistry which together display unique properties of nanomaterials with wide-range of applications [2]. The building blocks of nanotechnology are nanoparticles (NPs), whose particles dimensions ranged between 1 and 100 nm [3,4]. Due to the small size and bigger surface area NPs are different from bulk materials. Moreover, NPs and bulk materials also differs from each other in parameters such as physical strength, reactivity, electrical conductivity, optical features and magnetism [4,5]. These properties make the use of NPs in diverse fields such as energy, pharmaceutics, biomedical, cosmetics, textiles, food, and agriculture [6,7]. Studies in the past several years showed that nanotechnology has the ability to bring revolution in the field of agriculture, food and health sectors with the application of biosensors [8], plant growth regulators/promoters [9], food supplements [10], enhancement of plants and animals by genetic means [11,12], smart delivery agents for drugs, pesticides and fertilizers [13,14] and nanopesticides [15].

The nanoparticles are synthesized by chemical, physical and biological methods [16]. Although the synthesis of nanoparticles by physical and chemical ways is quite frequent but the use of toxic chemicals limits their applications in agriculture, food and health related applications [17]. However, the NPs synthesized by biogenic approach showed good polydispersity, dimension as well as stability. In addition, biological methods also allow the synthesis of NPs at physiological pH, temperature and pressure along with that, it is cost effective and ecofriendly [18,19].

The research in the area of nanotechnology is advancing, mainly due to valuable properties of nanomaterials and the products have been influencing all the major areas (Fig. 1). In this review, we have explored
In this study, silver nanoparticles (AgNPs) were synthesized using aqueous extract of *Nepeta deflersiana* plant. The prepared AgNPs (ND-AgNPs) were examined by ultraviolet-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscope (SEM), and energy dispersive spectroscopy (EDX). The results obtained from various characterizations revealed that average size of synthesized AgNPs was 33 nm and in face-centered-cubic structure. The anticancer potential of ND-AgNPs was investigated against human cervical cancer cells (HeLa). The cytotoxic response was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), neutral red uptake (NRU) assays, and morphological changes. Further, the influence of cytotoxic concentrations of ND-AgNPs on oxidative stress markers, reactive oxygen species (ROS) generation, mitochondrial membrane potential (MMP), cell cycle arrest and apoptosis/necrosis was studied. The cytotoxic response observed was in a concentration-dependent manner. Furthermore, the results also showed a significant increase in ROS and lipid peroxidation (LPO), along with a decrease in MMP and glutathione (GSH) levels. The cell cycle analysis and apoptosis/necrosis assay data exhibited ND-AgNPs-induced SubG1 arrest and apoptotic/necrotic cell death. The biosynthesized AgNPs-induced cell death in HeLa cells suggested the anticancer potential of ND-AgNPs. Therefore, they may be used to treat the cervical cancer cells.

1. Introduction

Nobel metal nanoparticles have attracted the interest of scientific community due to their fascinating applications in the field of biology, material science, medicine, etc [1]. Silver nanoparticles specifically have gained attention due to their unusual physiochemical [2] (chemical stability and electrical conductivity) and biological activities such as antibacterial, antifungal, anti-inflammatory, antiviral, antiangiogenesis, anticancer, and antiplatelet activities [3–5]. In addition, silver nanoparticles have been used in clothing [6], room spray, laundry detergent, wall paint formulation [7, 8], sunscreens, and cosmetics [9]. Silver nanoparticles also inhibit HIV-1 virus from binding to the host cells in vitro [10]. Although a wide variety of metal nanoparticle preparation methods such as UV radiation, laser ablation, lithography, aerosol technologies, and photochemical reduction are available [11–13], the focus is shifting towards green synthesis of nanoparticles, using bacteria [14], yeast [15], fungi [16], and plants [17]. Green synthesis of
Bacterial toxicity of biomimetic green zinc oxide nanoantibiotic: insights into ZnONP uptake and nanocolloid–bacteria interface†

Bilal Ahmed, Bushra Solanki, Almas Zaidi, Mohammad Saghir Khan and Javed Musarrat‡

This study was aimed to fill the critical gap of knowledge regarding the interaction between green zinc oxide nanoparticles (ZnONPs) and bacterial interface. Wurtzite phase ZnONPs with a band gap energy of 3.28 eV were produced by exploiting a simple and green biosynthesis method using an inexpensive precursor of A. indica leaf extract and zinc nitrate. ZnONPs were characterized using UV-Vis spectroscopy, XRD, FTIR, SEM, EDX, DLS, TEM, and zeta-potential analysis. The primary size obtained was 26.3 nm (XRD) and 33.5 ± 6.5 nm (TEM), whereas, the secondary size was found to be 287 ± 5.2 nm with −32.8 ± 1.8 mV ζ-potential denoting the physical colloid chemistry of ZnONPs. Crystallinity and the spherical morphology of ZnONPs were also evident with some sort of particle agglomeration. ZnONPs retained plant functional groups endorsing their hydrophilic character. The antibacterial and antibiofilm activity of ZnONPs was significant (p ≤ 0.05) and the MIC/MBC against most frequent clinical isolates of Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus ranged from 0.5 to 1.0 (MIC)/1.0 to 1.5 mg ml⁻¹ (MBC). The dissolution of ZnONPs to Zn²⁺ ions in a nutrient medium increased as a result of interaction with the bacterial surface and metabolites. Substantial surface binding of ZnONPs followed by intracellular uptake disrupted the cell morphology and caused obvious injury to the cell membrane. Interrupted bacterial growth kinetics, loss of cell respiration, enhanced production of intracellular ROS, and the leakage of the cytoplasmic content unequivocally suggested a strong interaction of ZnONPs with the exterior cell surface and intracellular components, eventually leading to cell death and destruction of biofilms. Overall, the results elucidated eco-friendly production of ZnONPs expressing a prominent interfacial correlation with bacteria and hence, prospecting the use of green ZnONPs as effective nanoantibiotics.

1. Introduction

The developments in manufacturing and increasing applications of nanomaterials, in recent times, have affected almost every domain of human life.¹ Metal and metal oxide nanoparticles (NPs) such as silver, gold, zinc oxide, iron oxide, copper oxide etc. are extensively used in a range of consumer goods. Recently, they have also been widely used in agriculture, medical, and pharmaceutical products.²–⁴ When desired, NPs have been synthesized using physical and chemical methods, for instance, microwave irradiation, thermal evaporati

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Chromosomal aberrations, cell suppression and oxidative stress generation induced by metal oxide nanoparticles in onion (Allium cepa) bulb†

Bilal Ahmed, Mohammad Shahid, Mohammad Saghir Khan and Javed Musarrat‡

There has been rapid increase globally in the production of functionally divergent nanoparticles in recent times. The uncontrolled discharge of such nanomaterials is a serious threat to the environment. We assess the impact of various-sized metal oxide nanoparticles (MONPs) on cell cycle progression and induction of oxidative stress in onions. Of these, CuO-NPs and TiO2-NPs significantly reduced the mitotic index (MI) by 28% and 17%, respectively, whereas Al2O3-NPs augmented the MI by 13% compared to untreated onion roots. The NPs internalization into the root tissues followed a dose dependent fashion. Also, several types of chromosomal aberration such as bridges, stickiness, vagrant, broken, and lag chromosomes were noticed. The reactive oxygen species activity of roots growing under CuO-NPs, Al2O3-NPs, and TiO2-NPs was significantly increased by 58, 30, and 10%, respectively. The superoxide dismutases activity (U g⁻¹ FW) of roots increased from 2.4 ± 0.4 (control) to 6.1 ± 0.8 (CuO-NPs), 4.1 ± 0.2 (Al2O3-NPs) and 2.9 ± 0.2 (TiO2-NPs), whereas, catalase activity (mnoles min⁻¹ g⁻¹ FW) was recorded as 18.5 ± 2.1 (CuO-NPs), 15 ± 11 (Al2O3-NPs) and 13.8 ± 1 (TiO2-NPs) against 11.4 ± 1 (control). The formazan formed due to superoxide (O₂⁻) reaction with nitroblue tetrazolium showed a dose dependent increase in roots treated with Al2O3-NPs and TiO2-NPs. Interestingly, under CuO-NPs exposure, the absorbance was considerably high at 200 µg ml⁻¹ which dropped at 2000 µg ml⁻¹ suggesting a clear attenuation of O₂⁻ by superoxide scavenging enzymes. The present findings provide base line data for better understanding of the mechanistic basis of phytotoxicity of MONPs to onion plants which can further be extended to other vegetable crops.

1. Introduction

Nanoparticles (NPs) have become an important material for several industrial, biomedical, and agricultural processes.¹ Moreover, the rapid advancement in nanotechnology, the high demand for engineered NPs, and the uncontrolled discharge of nanomaterials into the environment has led to adverse impacts on the environment. Additionally, the long latency period of NPs and their synergistic action has been found to adversely affect the health of both plants and humans.²,³ Despite this, NPs are frequently used due largely to their unique physico-chemical properties coupled with high surface area to volume ratio. For example, Al2O3-NPs are used as insulators and abrasive agents in the manufacturing of water resistant coatings on ships, as alloys, sensors, rocket fuels, personal care products, cosmetics, pharmaceuticals, toothpaste, optics, genomics, bioanalytical fields, and proteomics.⁵,⁷ Large amounts of NPs released from these products accumulate in terrestrial and water ecosystems and, following uptake, influence the organisms inhabiting such ecosystems.⁸ Among living organisms, plants in particular provide a very high surface area for NPs and therefore, NPs can be bio-sorbed by plants and accumulate in the food chain.⁹,¹⁰ Moreover, the trans-generational impact of NPs has been reported.¹¹ Cerium oxide NPs (10 mg l⁻¹) were found to transfer from one generation of tomato plants to the next, and second generation seedlings were weaker and smaller with slightly higher reactive oxygen species (ROS) content.¹¹ In addition to this, multi-generational exposure of plants to NPs has been found to reduce the accumulation of phosphorus, potassium, calcium, magnesium, and manganese.¹² Among various plant organs, root tips, being the first organ coming into direct contact with NPs, present an

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Toxicity assessment of metal oxide nano-pollutants on tomato (*Solanum lycopersicon*): A study on growth dynamics and plant cell death*

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**A B S T R A C T**

The present study for the first time demonstrated the interactions of metal oxide (MO) nano-pollutants (CuO and Al2O3-NPs) with tissues and cellular DNA of tomato plants grown in soil sand: silt: clay (667:190:143) and Hoagland-hydroponic system and assessed the hazardous effects of NPs on cell physiology and biochemistry. Results of SEM equipped with EDX revealed attachment of variably shaped CuO-NPs (18 nm) and Al2O3-NPs (21 nm) on roots, and internalization followed by translocation in plants by ICP-MS and TEM. Significant variations in foliage surface area, chlorophyll, proteins, LPO, and antioxidant enzymes were recorded. Roots and shoots accumulated 225.8 ± 8.9 and 70.5 ± 4 mgAl g⁻¹ DW, whereas Cu accumulation was 341.6 ± 14.3 (roots) and 146.9 ± 8.1 mgg⁻¹ DW (shoots) which was significant (p < 0.0005) as compared to control. The total soluble protein content in roots, shoots, and leaves collected from Al2O3-NPs treated plants increased by 120, 80, and 132%, respectively while in CuO-NPs treatments, the increase was 68 (roots), 36 (shoots), and 86% (leaves) over control. The level of antioxidant enzymes in plant tissues was significantly (p < 0.05) higher at 2000 μgml⁻¹ of MONPs over control. A dose-dependent increase in reactive oxygen species (ROS), biphasic change of lower and higher fluorescence in mitochondria due to dissipation of mitochondrial membrane potential (∆Ψm) and membrane defects using propidium iodide were observed. Comparatively, CuO-NPs induced higher toxicity than Al2O3-NPs. Perceptible changes in proteins (amide-I & II), cellulose, glucose, galactose and other carbohydrates were observed under FT-IR. The binding studies with TmDNA showed fluorescence quenching of EtBr-TmDNA and acridine orange-TmDNA complex only by CuO-NPs with -DG and +DH and ∆AS values. However, Al2O3-NPs induced lesser change in TmDNA conformation. Conclusively, the results are novel in better demonstrating the mechanistic basis of nano-phytotoxicity and are important which could be used to develop strategies for safe disposal of Al2O3-NPs and CuO-NPs.

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1. Introduction

Recent developments in nanotechnology and widespread applications of manufactured nanoparticles (NPs) have revolutionized the field of science and technology. The smaller size provides high reactivity which makes NPs suitable for use in biomedical, pharmaceuticals, electronics, defense, aerospace industries and agriculture fields (Ocsoy et al., 2013; Vittori Antisari et al., 2015; Rizwan et al., 2016). Among the MONPs, Al2O3-NPs and CuO-NPs are used in explosives, alloys, sensors, drug delivery, personal care products, catalysts, gas sensor, semiconductor devices, batteries, microelectronics, antimicrobial coatings, textiles, and food containers (Siddiqui et al., 2013; Rajeshwari et al., 2015; Ahmed et al., 2018). Due to ever increasing applications, the production of MONPs is likely to increase from 0.27 million tons (2012) to 1.663 million tons by 2020 (The Global Market for Metal Oxide Nanoparticles to 2020). Of these, 8–28%, 0.4–7.0%, and 0.1–1.5% are likely to enter into soils, water bodies and atmosphere, respectively (Keller et al., 2013; Rajput et al., 2018). Furthermore, the uncontrolled disposal of nanoparticles may also trigger their accumulation in the environment (Ma et al., 2010; Rastogi et al., 2017). The accumulation of NPs has however, caused human health problems (Siddiqui et al., 2013;...
Heavy metal induced oxidative damage and root morphology alterations of maize (Zea mays L.) plants and stress mitigation by metal tolerant nitrogen fixing Azotobacter chroococcum

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ABSTRACT

Heavy metals are one of the major abiotic stresses that adversely affect the quantity and nutritive value of maize. Microbial management involving the use of plant growth promoting rhizobacteria (PGPR) is a promising inexpensive strategy for metal clean up from polluted soils. Considering these, metal tolerant plant growth promoting nitrogen fixing rhizobacterial strain CAZ3 identified by 16srrRNA gene sequence analysis as Azotobacter chroococcum was recovered from metal polluted chilli rhizosphere. When exposed to varying levels of metals, A. chroococcum survived up to 1400 and 2000 µg mL\(^{-1}\) of Cu and Pb, respectively and expressed numerous plant growth promoting activities even under metal stress. Strain CAZ3 secreted 65.5 and 60.8 µg mL\(^{-1}\) IAA at 400 µg mL\(^{-1}\) each of Cu and Pb, respectively and produced siderophores, ammonia and ACC deaminase under metal pressure. The melanin extracted from A. chroococcum revealed metal chelating ability under EDX. Following application, strain CAZ3 enhanced growth and yield of maize grown both in the presence of Cu and Pb. The dry biomass of roots of inoculated plants grown with 2007 mg Cu kg\(^{-1}\) and 585 mg Pb kg\(^{-1}\) was increased by 28% and 20%, respectively. At 585 mg Pb kg\(^{-1}\), the bioinoculant also increased the kernel attributes. At 2007 mg Cu kg\(^{-1}\) strain CAZ3 enhanced the number, yield of kernels by 10%, 45% and 6%, respectively. Interestingly, strain CAZ3 significantly reduced the levels of proline, malondialdehyde and antioxidant enzymes in foliage. The roots of inoculated plants accumulated greatest amounts of metals compared to other organs. In kernels, the concentration of Pb was more as compared to Cu. The metal concentrations in roots, shoots and kernels, however, declined following CAZ3 inoculation. Copper and lead had substantial distortive impact on root and leaf morphology while cell death were visible under CLSM and SEM. Conclusively, A. chroococcum CAZ3 could be a most suitable and promising option to increase maize production in metal polluted soils despite the soils being contaminated with heavy metals.

1. Introduction

Maize (Zea mays L.), an edible flowering plant is cultivated preferably during spring and summer. Globally, maize, ranked as the third most important cereal crop after wheat and rice (Akontwubel et al., 2012) is used largely as food both by humans and animals (Lu et al., 2015). Maize provides carbohydrates, protein, minerals, vitamin B and iron in human diet (Olaniyan, 2015). Maize, like many other plants, however, suffers heavily by metals and eventually the growth and grain yield is greatly reduced (Aliu et al., 2013). Among metals, Pb for instance, is highly toxic and at excessive levels, slows down the germination rate and obstructs the height and biomass of maize plants (Ghani et al., 2016). Also, at elevated levels, it decreases cell division with brittle leaves and dark purple colour spots on plants (Imdad et al., 2017). Other toxic impact of Pb includes water imbalance, disturbances in nutrient uptake, disruption in photosynthetic process, altered enzymatic activities and overall hormonal imbalance (Sedzik et al., 2015; Ashraf et al., 2015). Copper (Cu) is another important metal which at lower concentration acts as essential micronutrient but at higher concentration it becomes highly toxic and inhibits photosynthesis (Dey et al., 2014), nutrient absorption, plant growth (Adrees et al., 2015) and ultimately cause cell death (Printz et al., 2016). Also, excessive amounts of Cu generates reactive oxygen species (ROS) leading to the oxidative damage of the plant cell (Liu et al., 2014). Due to these, there is urgent need to find solutions to the metal toxicity problems so that maize production continues even in soils contaminated with heavy metals. In this regard, certain beneficial bacteria aggressively colonizing the plant roots termed as plant growth promoting rhizobacteria...
Original Research Paper

ROS mediated destruction of cell membrane, growth and biofilms of human bacterial pathogens by stable metallic AgNPs functionalized from bell pepper extract and quercetin

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Abstract

Yellow pepper extract and quercetin (QDH) were used for YPE-AgNPs and Q-AgNPs fabrication. The AgNPs were thoroughly characterized using standard physico-chemical techniques and were found monodispersed, pleomorphic and had variable shape and size with a lattice fringe of 0.23 nm. YPE-AgNPs and Q-AgNPs revealed a characteristic SPR band at 438 nm and 431 nm. The XRD crystal size of YPE-AgNPs and Q-AgNPs was 10.16 and 12.20 nm while TEM analysis showed a size range of 5–40 and 1–25 nm. Bio-fabricated AgNPs remained stable for at least four weeks as the SPR did not deviate with time. FTIR data revealed functionalization of AgNPs by organics of reaction mixture. AgNPs had robust antibacterial and antibiofilm activity against ESbL Escherichia coli, Pseudomonas aeruginosa, and methicillin sensitive and resistant Staphylococcus aureus. Staining of isolates with fluorescent probes displayed the increased production of ROS and membrane permeability. AgNPs hampered EPS production, endorsed DNA leakage, and generated superoxide radicals. Time and concentration dependent experiments demonstrated a consistent decrease in bacterial growth. Structural changes viz. irregular margins, distortion, depressions/indentations and shrinkage of cells were obvious under SEM. AgNPs due to strong antibacterial activity could be exploited as a supplement with antibiotic drugs to control resilient human infections.

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1. Introduction

The constantly increasing use of nanoparticles in biosensor development, medical diagnosis, pharmaceutical and therapeutics is gaining a great momentum [1,2]. And hence, the synthesis of novel nanoparticles with fixed shape and size has been widely used to develop some novel antibacterial drugs for effective management of both plants and human bacterial pathogens [3]. The control over size and shape of nanoparticles alters their optical, chemical and biological traits [4]. Also, the greater surface area to volume ratio of nanoparticles relative to their bulk counterpart parts, their ability of absorption in visible region and reduction in particle size increases their therapeutic value against a range of diseases with minimal side effects [5,6]. Considering the importance of nanoparticles, several physical, chemical and biological methods have been developed and adopted to synthesize metal nanoparticles [7,8]. Of these, chemical methods which employ reducing agents such as sodium borohydride have been found expensive and highly toxic [9]. Due to these, there is an urgent need to find an inexpensive and environmentally friendly strategy to produce safe, inexpensive and effective therapeutic nanoparticles. In this regard, plant extracts and phenolic compounds purified from plants have been found as a valuable alternative to hazardous physico-chemical methods used for nanoparticles generation [10]. The eco-friendly green synthesis involves phytochemicals for biomolecular reduction with better control over shape and size without showing any toxicity [11]. Plant and plant derived compounds mediated synthesis of nanoparticles offer certain important advantages including- (i) it is cost effective and eco-friendly (ii) method can be easily scaled up for pilot scale production and (iii) it does not require high energy, pressure, temperature and use of toxic chemicals. Among plant metabolites, flavonoids, terpenoids, proteins, reducing sugars and phenolic acids have been found to play key roles in the reduction of metal ions leading to the formation and stabilization of nanoparticles [12,13].

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Assessment of toxic impact of metals on proline, antioxidant enzymes, and biological characteristics of *Pseudomonas aeruginosa* inoculated *Cicer arietinum* grown in chromium and nickel-stressed sandy clay loam soils

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**Abstract** Considering the heavy metal risk to soil microbiota and agro-ecosystems, the study was designed to determine metal toxicity to bacteria and to find metal tolerant bacteria carrying multifarious plant growth promoting activities and to assess their impact on chickpea cultivated in stressed soils. Metal tolerant strain SFP1 recognized as *Pseudomonas aeruginosa* employing 16S rRNA gene sequence determination showed maximum tolerance to Cr (400 μg/ml) and Ni (800 μg/ml) and produced variable amounts of indole acetic acid, HCN, NH₃, and ACC deaminase and could solubilize insoluble phosphates even under Cr (VI) and Ni stress. Metal tolerant *P. aeruginosa* reduced toxicity of Cr (VI) and Ni and concomitantly enhanced the performance of chickpea grown under stressed and conventional soils. At 144 mg Cr kg⁻¹, the measured parameters of a bacterial strain was significantly enhanced, but it was lower compared to those recorded at 660 mg Ni kg⁻¹. The strain SFP1 demonstrated maximum increase in seed yield (81%) and grain protein (16%) at 660 mg Ni kg⁻¹ over uninoculated and untreated control. Stressed plants had more proline, antioxidant enzymes, and metal concentrations in plant tissues. *P. aeruginosa*, however, remarkably declined the level of stress markers (proline and APX, SOD, CAT, and GR), as well as with Cr (VI) and Ni uptake by chickpea. Conclusively, *P. aeruginosa* strain SFP1 due to its dual metal tolerant ability, capacity to secrete plant growth promoting regulators even under metal stress and potential to mitigate metal toxicity, could be developed as microbial inoculant for enhancing chickpea production in Cr and Ni contaminated soils.

**Keywords** Metals · PGPR · Bioremediation · Chickpea · Antioxidant enzymes

**Introduction**

Rapid industrialization and unguided release of heavy metals from various anthropogenic sources into the environment is a growing concern worldwide (Singh et al. 2014). Among heavy metals, some metals for example, Cu, Zn, and Fe at micronutrient levels are required for several plant physiological processes but at higher concentrations become toxic (Wani and Khan 2010). However, other metals like Ag, Hg, Cd have no biological functions and are toxic even at extremely low concentrations (Paz-Ferreiro et al. 2014). The toxicity of numerous metals to many soil microbiota (Xie et al. 2016) including plant growth promoting rhizobacteria (PGPR) and protein rich consumable legumes (Kaur and Nayar 2013) including chickpea is reported (Imtiaz et al. 2016). Chickpea among legumes has vital dietary importance in developing countries and ranks third in production among grain legumes in the world. Recently, the use of sewage water containing different metals in chickpea cultivation has increased considerably which in long-term has resulted in poor growth and lower
Glyphosate induced toxicity to chickpea plants and stress alleviation by herbicide tolerant phosphate solubilizing *Burkholderia cepacia* PSBB1 carrying multifarious plant growth promoting activities

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Abstract
In this study, strain PSBB1 isolated from *Vicia faba* rhizosphere was identified as *Burkholderia cepacia*, by 16S rDNA sequence analysis and characterized. Strain PSBB1 tolerated glyphosate up to 3200 μg ml⁻¹ and produced IAA (81.6 μg ml⁻¹), ACC deaminase (69.3 mg⁻¹ protein h⁻¹), SA (39.3 μg ml⁻¹) and 2,3-DHBA (26.6 μg ml⁻¹), solubilized insoluble P (50.8 μg ml⁻¹) and secreted 29.4 μg ml⁻¹ exopolysaccharides, which decreased with increasing concentrations of glyphosate. Cell damage following glyphosate application was visible under SEM and CLSM. The phytotoxicity of glyphosate on chickpea was variable but significant. *B. cepacia* mitigated toxicity and enhanced the size, dry matter, symbiosis, seed attributes and nutritional contents of chickpea. Further, *B. cepacia* strain PSBB1 declined the levels of CAT, POD, APX and GPX and MDA contents at 4332 μg kg⁻¹ soil glyphosate. Proline also increased under glyphosate stress but declined in *B. cepacia* inoculated plants. The ability to tolerate higher concentration of glyphosate, the capacity to secrete plant growth regulators even under herbicide stress and potential to reduce the level of proline and antioxidant enzymes makes *B. cepacia* as an interesting choice for enhancing chickpea production in soils contaminated even with herbicides.

Keywords Chickpea · Herbicide toxicity · *Burkholderia cepacia* · Proline · Antioxidant enzymes · Bioremediation

Introduction
Herbicides are frequently and abruptly used in intensive cropping systems for optimizing crop production. Owing to widespread and inadvisable application, major portion of the herbicides used in excess amount, however, persist within soils (Curran 2016). Following accumulation within soils and later on uptake by plants, herbicides cause toxicity to many crops including legumes (Ugbe et al. 2016). Among legumes, chickpea is considered important due to its protein rich nutritional value. In addition, it is severely affected due to its inability to compete with weeds as it has limited growth rate and leaf area which grow slowly during initial growth stages (Goud et al. 2013). Moreover, it needs wider spacing during cultivation which facilitates crop weed competition which as a result pose a serious threat to crop quality and production unless it is controlled effectively. However, to eradicate weeds from cultivable fields, suitable weed control strategies involving mechanical practices, crop rotations, hand weeding and application of herbicides are available and practiced in agricultural practices. The herbicides besides exhibiting inhibitory effects also cause threat to the existence and physiological functions of rhizobacteria (Nandula and Tyler 2016) and, consequently, indirectly affects the soil fertility (Bitew and Alemayehu 2017). Glyphosate, a broad spectrum systemic herbicide which belongs to organophosphorus family is applied to destroy weeds, especially annual broadleaf weeds and grasses which in turn limit the growth of crops. While evaluating the impact of high concentration of glyphosate on nitrogenase activity of numerous rhizobial strains, Zablotowicz and Reddy (2007) observed that herbicides considerably declined nitrogenase activity of rhizobia. As a consequence, the symbiotic events leading to nodule formation and root morphogenesis of the test plants were drastically diminished (Adami et al. 2017). Similarly, the lethal impact of certain herbicides like pendimethalin, chlormuron, propaquizafop, oxyfluorfen and imazethapyr on...
Evaluation of microbiological management strategy of herbicide toxicity to greengram plants

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Bioremediation
Growth promotion

ABSTRACT

In order to circumvent the problems of herbicidal toxicity, tolerant N2 fixing and phosphorus solubilizing bacterial strains were isolated and identified. Among 20 bacterial isolates, Azotobacter sp. strain AZ1 survived 2400, 3200, and 1600 μg/mL while Bacillus sp. strain PSB2 tolerated up to 1600, 2400 and 1600 μg/mL glyphosate, quizalofop and metribuzin, respectively. Under in vitro conditions, these bacterial strains secreted IAA, siderophores, exopolysaccharides, ammonia and transformed inorganic P into organic P even under herbicides stress. SEM and CLSM images revealed a clear toxic impact of herbicides on bacterial cells above tolerance limit. Phytotoxicity to greengram plants increased with increasing concentrations of herbicides. Herbicide tolerant Azotobacter sp. strain AZ1 and Bacillus sp. strain PSB2 when used as inoculant, substantially reduced the herbicidal toxicity to greengram. For instance, strain AZ1 increased the length of roots (10%) and shoot (6%), dry biomass of root (28%) and shoot (6%), different symbiotic parameters like nodule number (6%), nodule dry biomass (8%), LHb (15%), photosynthetic pigments and seed yield (39%), whereas, Bacillus sp. strain PSB2 enhanced the measured parameters by 12%, 13%, 23%, 21%, 4%, 6%, 22%, 5% and 27%, respectively relative to positive control (1444 μg/kg glyphosate). Additionally, proline in shoot tissues declined rapidly in bio-inoculated plants. Conclusively, the microbial cultures resulted in better management of herbicidal toxicity to greengram plants. And hence, Azotobacter sp. strain AZ1 and Bacillus sp. strain PSB2 could be recommended for use as an effective and inexpensive microbial inoculant/biofertilizer to augment the production of greengram in herbicide contaminated soils.

1. Introduction

Pesticides application for protecting crops from pest’s damage has been increased alarmingly in recent times. The pesticides however when accumulates beyond certain threshold level, alter soil microbial composition (Zaller et al., 2016), disrupts soil fertility (Mukherjee et al., 2016), and other physicochemical properties of soil (Usman et al., 2017). On the contrary, due to weed competition, the production of legumes suffers heavily. Henceforth, to control weeds and subsequently to augment crop production, herbicides are applied regularly (Jiddimani, 2017). Amongst legumes, greengram is a highly nutritious grain legume cultivated in tropics and provides proteins (19–28%), minerals (0.18–0.21%) and vitamins. It is largely used as human staple food in many countries including India (Hari et al., 2017). Also, when applied, herbicides had variable effects on greengram production (Gupta et al., 2017). These toxic problems associated with herbicide usage, however, can be circumvented by applying naturally occurring soil microflora. Among miscellaneous microbial communities, plant growth promoting rhizobacteria (PGPR) have been found to reduce the agrochemical toxicity by different mechanisms (Azubuike et al., 2016) and has therefore, received considerable attention by the agronomists than the microbiologists. To this end, the PGPR offers a greatly viable and economically inexpensive option for immensely safe detoxification/removal of toxic chemicals from contaminated sites (Akbar and Sultan, 2016). In this regard, Azotobacter (Gram negative), an aerobic and free-living, nitrogen-fixing bacteria distributed over a range of agro-ecological niches have been reported to degrade /detoxify the agrochemicals leading eventually to a safe and viable solution to contamination problems (Gauri et al., 2012). Bacillus (Gram positive and aerobic) is yet another agronomically important soil bacterium which has the ability to solubilize inorganic form of P into organic form and is capable of degrading many toxic pesticides including herbicides to non-toxic forms (Geed et al., 2017). Apart from these, Azotobacter (Gotandapani et al., 2017) and Bacillus (Lastochkina et al., 2017) inoculation have shown beneficial effects against many field crops by supplying N to plants and through secretion/production of growth

Abbreviations: MIC, Minimum inhibitory concentration; IAA, Indole-3-acetic acid; HCN, Hydrogen cyanide; DAS, Days after sowing; LHb, Leghaemoglobin

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Cellular destruction, phytohormones and growth modulating enzymes production by \textit{Bacillus subtilis} strain BC8 impacted by fungicides

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\textbf{A R T I C L E   I N F O}

Keywords: Fungicides \textit{B. subtilis} Oxidative damage Cellular toxicity Membrane integrity bioactive molecules

\textbf{A B S T R A C T}

\textit{In vitro} experiments were performed to ascertain the impact of kitazin, hexaconazole, metalaxyl and carbendazim on growth behaviour, enzymatic profile, ultrastructure, cell permeability and bioactive molecules of \textit{B. subtilis} strain BC8. Strain BC8 isolated from \textit{Brassica oleracea} rhizosphere was characterized and identified as \textit{Bacillus subtilis} by 16S rDNA sequencing (Accession no. MG028650) technique. Strain BC8 was unambiguously chosen due to its high tolerance capability to various fungicides and substantial production of plant growth regulators. The biomarker enzymatic assays (lipid peroxidation, lactate dehydrogenase) and oxidative stress (catalase) induced by fungicides exhibited significant (p < 0.05) toxicity of fungicides toward strain BC8. Fungicides caused the cellular/ultrastructural damage and reduced the viability of strain BC8 as clearly revealed under scanning (SEM), high resolution transmission (HR-TEM) and confocal laser scanning (CLSM) microscopy. As the concentration of fungicides increased, a gradual drop in the plant growth promoting traits of \textit{B. subtilis} strain BC8 was observed. Kitazin at 2400 μg mL\textsuperscript{-1}, hexaconazole at 1500 μg mL\textsuperscript{-1}, metalaxyl at 1200 μg mL\textsuperscript{-1} and carbendazim at 1200 μg mL\textsuperscript{-1} decreased the IAA production by 35 (48.3 μg mL\textsuperscript{-1}), 27 (51.5 μg mL\textsuperscript{-1}), 39 (43.6 μg mL\textsuperscript{-1}) and 47% (37.3 μg mL\textsuperscript{-1}), respectively, over control (71.3 μg mL\textsuperscript{-1}), while, α-ketobutyrate was declined by 51 (29.6), 56 (26.2), 61 (22.8) and 68 (19)%, respectively, over untreated control (59.9 mg protein \textsuperscript{-1} h\textsuperscript{-1}). Also, with increase in the concentration of fungicides there was a significant decrease in plant nutrient (P); the maximum being (19.6 μg mL\textsuperscript{-1}) observed at 1500 μg mL\textsuperscript{-1} hexaconazole with consequent drop in pH (from pH 6.4 to 4.2). The current findings clearly suggest that despite injury, \textit{B. subtilis} maintained secreting active biomolecules and this property makes this organism truly indispensable for enhancing crop production under fungicide stressed conditions.

1. Introduction

Fungicides in intensive agricultural practices are frequently used to control/manage numerous soil borne phytopathogens concurrently optimizing crop production [1]. It is estimated that approximately 2.8 million tonnes of the pesticides are used worldwide for enhancing crop production each year which has threatened the very sustainability of agro-ecosystem [2,3]. Whereas, pesticides including fungicides are essential for plants to relieve the phytopathogens pressure [4]. The supply of active biomolecules like phytohormones [5] and plant nutrients (P and N) on the other hands are equally important to facilitate plant growth. However, the continued and hysterical application of the synthetic fungicides in crop production has led to the destruction of soil fertility [6] and decline both in the quantity and quality of foods [7] which in turn via food chain adversely affects the human health [8]. In this regard, fungicides have shown inhibitory effect on the composition and functions of beneficial soil bacteria [9] and edible crops [10]. As a result, the microbial community structure and associated soil chemistry generally referred to as soil fertility are lost [11]. For instance, hexaconazole, a broad spectrum and synthetic fungicide have been found to negatively affect the soil microbial biomass, microbial physiology, respiratory activity, bacterial abundance and community structure [12]. Also, other fungicides like, mancozeb and dimethomorph are reported to reduce massively the number of soil microorganisms [13], microbial ecology and enzyme activity [14]. While, considering these and other related available data, many scientists have attempted to assess the lethal impact of fungicides on survival and associated plant growth regulating activities of plant growth promoting rhizobacteria (PGPR). In this context, the effect of varying doses of organochlorine pesticides on plant growth promoting traits of phosphate solubilizing \textit{Paenibacillus} sp. strain ITI15SM08 isolated from rhizosphere soil, has recently been reported [15]. Several xenobiotic compounds including agrochemicals have been found to generate numerous free radicals which increases the cellular
Toxicity of fungicides to *Pisum sativum*: a study of oxidative damage, growth suppression, cellular death and morpho-anatomical changes

Mohammad Shahid, Bilal Ahmed, Almas Zaidi and Mohd Saghir Khan

Considering the fungicidal threat to the sustainable agro-environment, the toxicological impacts of three fungicides, namely kitazin, hexaconazole and carbendazim, on the biological, chemical and morpho-anatomical changes of peas were assessed. Fungicide applications in general caused a slow but gradual reduction in growth, symbiosis and yields of peas, which, however, varied appreciably among species and concentrations of the three fungicides. Of the three fungicides, carbendazim had the most lethal effect, in which it delayed seed germination and also diminished the overall pea growth. Carbendazim at 3000 \( \mu g \) \( kg^{-1} \) maximally reduced the germination, SVI, size of roots and shoots and total dry matter accumulation in roots, shoots and whole plants distinctly by 40%, 84%, 72%, 73%, 68%, 75% and 73% (\( p \leq 0.05 \)), respectively. Hexaconazole at 120 \( \mu g \) kg\(^{-1} \) significantly (\( p \leq 0.05 \)) declined total chlorophyll, carotenoids, grain yields, grain protein, root P and shoot N by 19%, 28%, 46%, 69%, 48% and 51%, respectively, over the control. The synthesis of stress biomarkers and oxidative stress were increased with increasing dosage rates of fungicides. Proline content in roots, shoots, leaves and grains, MDA, electrolyte leakage and \( H_2O_2 \) of plants grown in soil treated with 288 \( \mu g \) kg\(^{-1} \) kitazin were increased significantly (\( p \leq 0.05 \)) by 73%, 52%, 41%, 24%, 59%, 40% and 27%, respectively, relative to the control. Antioxidant defence enzymes were greater in pea foliage. The SEM and CLSM images revealed an obvious alteration in root tips, enhanced cellular damage and cell death when plants were raised under fungicide stress. Also, morpho-anatomical variations in fungicide-treated foliage were visible in the SEM images. Overall, the present study suggests that a careful and secure strategy should be adopted before fungicides are chosen for enhancing pulse production in different agro-climatic regions.

1. Introduction

Pea (*Pisum sativum* L.) is one of the most widely cultivated crops and is grown both as a vegetable and as a pulse crop. Globally, it is grown in an area of 1.1 million ha with a total production of 9.2 million tonnes with a productivity of 8.35 tonnes per ha.\(^1\) Nutritionally, it contains high concentrations of protein, carbohydrates, vitamins and minerals (Fe, Ca, K and P), which make peas a valuable human dietary component.\(^2\) Also, due to low fats, sodium and cholesterol, it is used to prevent cardiovascular diseases.\(^3\) When grown in different parts of the world, this crop faces many challenges from weeds and insects,\(^4\) phytopathogens\(^5\) and environmental variables.\(^6\) Chief among these are the diseases caused by various phytopathogens that can lead to severe losses in crop yields.\(^5\) So, to protect peas from fungal damage and consequently to maintain their nutritional composition, various synthetic fungicides,\(^7\) for example, carbendazim, mancozeb, kitazin, hexaconazole, thiram, are applied indiscriminately in farming practices.\(^8\) The majority of these fungicides when applied, however, persist in soil and become non-biodegradable.\(^9\) Such fungicides in turn destruct the soil fertility,\(^10\) leading eventually to losses in the growth, symbiotic attributes and yields of legumes.\(^11\) Some fungicides even abolish nodulation and the \( N_2 \)-fixation processes of several grains and forage legumes. For instance, hexaconazole, a broad-spectrum systemic fungicide, is used to manage phytopathogenic fungi\(^12\) on the one hand, while it negatively affects the BNF, ureide levels, \( N \) transformation and overall performance of leguminous plants on the other hand.\(^13,14\) Also, it decreases chlorophyll and carotenoid pigment formation by legumes, for example, *Phaseolus vulgaris*.\(^15\) In yet another study, the fungicide pyrimorph has been found to strongly inhibit the electron-transport reactions of chloroplasts and thus damages the physiological machinery of whole plants.\(^16\) These fungicides act as multiple site inhibitors and have various types of toxic action on cells, for example chelation and the formation of mixed disulfide bonds transport across the membranes. Dialkyldithiocarbamates inhibit a widespread fungal enzyme, but the scheme of pyruvic dehydrogenase is predominantly delicate to such type of fungicides.\(^17\) Also, these anti-fungal compounds...
Differential surface contact killing of pristine and low EPS Pseudomonas aeruginosa with Aloe vera capped hematite (α-Fe₂O₃) nanoparticles

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ABSTRACT

Biogenic hematite (α-Fe₂O₃) nanoparticles (NPs) of average size < 10 nm were synthesized using green approach with Aloe vera extract (ALE). The aim of the study was to assess the protective effect of extracellular polymeric substances (EPS) against antibacterial and antibiofilm activities of ALE-α-Fe₂O₃NPs in normal EPS producers (pristine) and experimentally modified (low-EPS) Pseudomonas aeruginosa (P. aeruginosa) cells and the mechanism of cell killing. Formation of ALE-α-Fe₂O₃NPs has been validated by X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and Fourier-transformed infrared spectroscopy (FTIR) analysis. The FTIR data suggested the possible role OH group bearing organic compounds of ALE in metal reduction and nucleation of NPs. Gas Chromatography-Mass spectroscopy (GC–MS) analysis revealed the presence of oxime-methoxy-phenyl, ethanone 1-phenyl, hexadecanoic acid, cyclohexanol 2,6-dimethyl, tetracontane, stigmast-5-en-3-ol, cyclohexanol 2,6-dimethyl, and cyclohexasiloxane dodecamethyl on the surface of ALE-α-Fe₂O₃NPs. Cell viability assay and SEM imaging revealed significantly greater bacteriostatic and/or bactericidal effect of ALE-α-Fe₂O₃NPs in low EPS cells compared to pristine cells or bare-α-Fe₂O₃NPs. This is attributed to thinner protective layer of EPS around the low EPS cells, and higher dispersibility and stability of ALE-α-Fe₂O₃NPs. Absorption of ALE-α-Fe₂O₃NPs and bare-α-Fe₂O₃NPs on EPS surface and within EPS matrix was ascertained by atomic absorption spectroscopy (AAS). The results suggest differential internalization of ALE-α-Fe₂O₃NPs and bare-α-Fe₂O₃NPs in P. aeruginosa cells. The flow cytometry (FCM) results exhibited increased intracellular granularity in low EPS (18.94%) as compared with pristine (10.94%) cells, which signifies the greater internalization of ALE-α-Fe₂O₃NPs. Moreover, the proportionate increase in intracellular ROS generation in low EPS (20.47%) via-a-vis pristine (7.56%) cells was observed. Overall, the results elucidate that ALE-α-Fe₂O₃NPs-bacterial interaction leads to attachment of NPs to EPS surface, migration within the EPS matrix and penetration into cell, which eventually results in growth inhibition due to intracellular ROS activity. Owing to significant antibacterial and antibiofilm activities, ALE-α-Fe₂O₃NPs may serve as a good candidate for clinical management of extended spectrum beta lactamases (ESBL) positive P. aeruginosa.

1. Introduction

Development of environment friendly, cost-effective and easily scalable method is a hallmark of biogenic green approach for nanomaterials synthesis [1]. Ubiquitous availability of plant materials, and their innate redox reactions without involving any extraneous hazardous chemicals have prompted nanotechnologists to opt green approach for nanoparticles (NPs) synthesis [2,3]. Most of the physical and chemical methods used in the NPs synthesis are known to employ the toxic chemicals as reducing and stabilizing agents, organic solvent or non-biodegradable agents [3], which may pose environmental risks due to use of hazardous chemicals [1]. Recently, Hasan et al. [4] have highlighted the several merits of biogenic organic entities as potential chemical reactors for fabrication and surface functionalization of biocompatible NPs. Moreover, the association of phytocompounds with metallic and metal oxide particles during nucleation provides an enhanced stability and improved dispersity in aqueous media vis-a-vis the bare surface synthetic counterparts [5]. Plant extracts possess a variety of potent antioxidants such as polyphenols [6], reducing sugars [7], nitrogenous bases [7], and amino acids [8], which can reduce metal ions in a metal salt solution [9]. A plethora of reports on NPs synthesis via bio-inspired route using green extracts of Aloe vera [10], Camellia sinensis [11], Emblica Officinalis [12], Hibiscus rosasinensis [13] and Eucalyptus globulus [14] are available in literature. The Aloe vera plants

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Molecular cloning and characterization of a novel vip3-type gene from *Bacillus thuringiensis* and evaluation of its toxicity against *Helicoverpa armigera*

Showkat Ahmad Lone, Abdul Malik, Jasdeep Chatrath Padaria

**Abstract**

Vegetative insecticidal proteins (Vips) represent the second generation of insecticidal trans-genes that will complement the *Bacillus thuringiensis* delta endotoxins in future. A new vip3A gene was cloned from the promising native isolate, *B. thuringiensis* JK37 obtained from the soils of maize field. The entire coding sequence of the gene (2370 bp) was amplified and cloned into pET28a(+) expression vector. The deduced amino acid sequence of the vip3A gene revealed variation of several amino acid residues with that of the known vip3A genes and this gene was designated as vip3Aa61 by the *B. thuringiensis* nomenclature committee. The recombinant pET28a(+)--vip3Aa61 was transformed and expressed in *Escherichia coli* strain BL21 (DE3) under the control of T7 promoter. SDS-PAGE and Western blot analysis confirmed the expression of an 89 kDa protein. Insect bioassays with 2nd instar larvae of *Helicoverpa armigera*, one of the most notorious pest affecting various crops including cotton and chick pea displayed toxicity. The toxicity of Vip3Aa61 was expressed as mean lethal concentration (LC50), which was 169.63 ng cm−2. The novel vip3A gene may be used for the construction of transgenic plants expressing insecticidal protein for the control of lepidopteran insect pests.

**1. Introduction**

*Bacillus thuringiensis* (*Bt*) is the most successfully used biopesticide worldwide. The primary insecticidal armoury of *B. thuringiensis* are the crystalline proteins (Cry and Cyt) [1], which are toxic not only against insects of different orders but also against certain nematodes [2,3]. In addition to the crystalline proteins that are synthesized during sporulation phase, certain strains of *B. thuringiensis* also produce vegetative insecticidal proteins (Vips) during the vegetative phase of their growth [4]. Vips do not share amino acid homology with the crystalline proteins but do accord insecticidal property to the bacterium against certain insect pests [5,6]. As of July 2017, more than 100 Vips have been documented and they have been classified into four different classes viz. Vip1, Vip2, Vip3, and Vip4 [7]. Vip1 and Vip2 are binary toxins active against coleopteran [8] and hemipteran insects [9], while Vip3 toxins are effective against various lepidopteran pests [4]. Vip3 class is the most extensively studied among the Vips consisting of three main classes (Vip3A, Vip3B and Vip3C) and thirteen subclasses (Vip3Aa to Vip3Aaj, Vip3Ba, Vip3Bb and Vip3Ca) [7]. Vip3 toxins exhibit varying levels of insecticidal activity against a range of economically important lepidopteran pests [10]. Despite high degree of amino acid sequence identity, differences in insecticidal activity even among the subtypes of the particular Vip3 proteins have been observed [4,11,12]. Moreover, certain Vip3A subtypes did not show insecticidal activity against any lepidopteran insects [4,13,14].

It has been shown that Vip proteins differ in mechanism of action from the Cry proteins, particularly with respect to the receptor binding and ion channel properties, and both of them do not compete for binding sites [14–16]. Additionally, very low levels of cross-resistance between Vip3A and Cry1 proteins have been reported [17–19]. These useful properties make Vip3 proteins competent candidates for broadening the host range of *B. thuringiensis* and for coping up with the problem of insect resistance to Cry proteins [15,20,21].

*B. thuringiensis* JK37 is a native isolate obtained from maize fields at Kalgi of Kashmir valley, India. *In vitro* bioassays have demonstrated that this strain is highly toxic to *Helicoverpa armigera* [22], an economically important polyphagous pest. PCR screening of the JK37 revealed that the strain harbors vip3Aa gene. Considering the importance of Vip3A proteins in terms of their insecticidal activity, complete coding sequence (CDS) of vip3Aa61 gene from JK37 was cloned and expressed in...
Pendimethalin induces oxidative stress, DNA damage, and mitochondrial dysfunction to trigger apoptosis in human lymphocytes and rat bone-marrow cells

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Abstract
Pendimethalin (PM) is a dinitroaniline herbicide extensively applied against the annual grasses and broad-leaved weeds. There is no report available on PM-induced low-dose genotoxicity in human primary cells and in vivo test models. Such data gap has prompted us to evaluate the genotoxic potential of PM in human lymphocytes and rats. PM selectively binds in the minor groove of DNA by forming covalent bonds with G and C nitrogenous bases, as well as with the ribose sugar. PM induces micronucleus formation (MN) in human lymphocytes, indicating its clastogenic potential. Comet assay data showed 35.6-fold greater DNA damage in PM (200 μM)-treated human lymphocytes. Rat bone-marrow cells, at the highest dose of 50 mg/kg b w/day of PM also exhibited 10.5-fold greater DNA damage. PM at 200 μM and 50 mg/kg b w/day induces 193.4 and 229% higher reactive oxygen species generation in human lymphocytes and rat bone-marrow cells. PM-treated human lymphocytes and rat bone-marrow cells both showed dysfunction of mitochondrial membrane potential (ΔΨm). PM exposure results in the appearance of 72.2 and 35.2% sub-G1 apoptotic peaks in human lymphocytes and rat bone-marrow cells when treated with 200 μM and 50 mg/kg b w/day of PM. Rats exposed to PM also showed imbalance in antioxidant enzymes and histological pathology. Overall, our data demonstrated the genotoxic and apoptotic potentials of PM in human and animal test models.

Keywords Pendimethalin · Pesticide · Genotoxicity · Apoptosis · Oxidative stress · DNA damage

Introduction
Pendimethalin (PM) is a dinitroaniline pre-emergence herbicide widely used for the selective control of annual grasses and broad-leaved weeds in several crops (Tomlin 1994). PM interrupts plant cell division, chromosome separation, and cell wall formation (Richardson and Gangolli 2007). PM and its formulations have been manifested as soil, ground water, and air contaminants (Coscollà et al. 2017; Galli et al. 2011; Thomatou et al. 2013). Residue analysis of 180 pesticides from 80 different grain samples showed an elevated level of PM (0.11 mg/kg) in cereals, as compared to the assigned value of 0.108 mg/kg (Lozowicka et al. 2014). PM from soil may disperse through runoff, evaporation, drift and leaching (Frimmel and Hettich 1993; Mohan et al. 2007; Strandberg and Scott-Fordsmand 2004). Recent investigation on the temporal variation of atmospheric pesticide levels in the center region of France revealed that PM (0.13–117.32 ng m⁻³) were among...
An improved method of DNA preparation for PCR-based detection of *Brucella* in raw camel milk samples from Riyadh region and its comparison with immunological methods

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Abstract
Brucellosis a zoonotic disease is endemic in Saudi Arabia especially in the Najed region due to the customary consumption of raw milk. For a detailed investigation of brucellosis in camel milk, 80 samples were collected from Riyadh region. Twenty-four and sixteen percentage of the samples were positive for *Brucella* when tested with Rose Bengal test and ELISA, respectively. Genomic DNA from the milk samples was prepared using a modified method presented in this study. *Brucella* was detected in camel milk using three different primer sets targeting *Brucella* 16S rRNA gene, *omp*2, and *IS*711 genes. Among the three primer sets, an F4/R2 set of primers targeting *Brucella* 16S rRNA gene was most sensitive and yielded PCR products from nine milk samples. Finally, the 16S rRNA gene sequences of these isolates confirmed the contamination of *Brucella* spp. in nine milk samples. This study, therefore, suggests a high incidence of *Brucella* in camels of Riyadh.

Practical applications
This manuscript describes a modified method for the isolation of DNA from camel milk. The DNA was subsequently used successfully for the amplification of various genes for detecting *Brucella*. Furthermore, there was no report from Riyadh region on the detection of *Brucella* from camel milk. This report, therefore, points out toward an important health issue associated with consumption of raw camel milk. If published, this report will be helpful in educating the local population about the health risk associated with the consumption of raw milk.

1 | INTRODUCTION

Brucellosis a global zoonotic disease (Dean, Crump, Greter, Schelling, & Zinsstag, 2012; Pappas, Papadimitriou, Akritidis, Christou, & Tsianos, 2006) is a matter of special concern to Saudi Arabia where the incidence in some region is as high as 8–8.8% (Ahmed & Ahmed, 2009; Memish, 2001). Dean et al. (2012) have reviewed that the incidence of brucellosis in the kingdom is much higher than other countries in the region. According to some estimates, more than 8,000 cases per year are reported to public health authorities (Memish & Mah, 2001). The incidence is particularly higher in rural areas due to the nomadic tradition of consuming raw milk (Wernery, 2014). Surprisingly, very few systematic reports on brucellosis are available from the Kingdom, central Asia, and Sub-Saharan Africa (Al-Freihi et al., 1986; Dean et al., 2012; Kiel & Khan, 1987). Although reports on human brucellosis from different regions of Saudi Arabia are available almost no report on camel brucellosis, one of the primary sources of the disease, were found in the literature from the region (Ahmed & Ahmed, 2009; Al-Sekait, 2000; Gwida et al., 2012). Brucellosis is caused by *Brucella* a genus belonging to Alphaproteobacteria that currently comprises of six species (Moreno, Cloeckaert, & Moriyón, 2002). The two most prevalent species causing brucellosis in the Saudi Arabia are *Brucella melitensis* and *B. abortus* (Al-Eissa, 1999; Madkour, Mohamed, Talukdar, & Kudwah, 1985). It has also been demonstrated that the camels are sensitive to these two species of *Brucella* (Cooper, 1991). Therefore, it is both urgent and necessary to monitor the disease in the region, especially by controlling the source of the pathogen. Developing improved methods for economic, easy, and accurate detection of *Brucella* in camel or...
Biotoxic impact of heavy metals on growth, oxidative stress and morphological changes in root structure of wheat (Triticum aestivum L.) and stress alleviation by Pseudomonas aeruginosa strain CPSB1

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Cellular Damage, Plant Growth Promoting Activity and Chromium Reducing Ability of Metal Tolerant *Pseudomonas aeruginosa* CPSB1 Recovered from Metal Polluted Chilli (*Capsicum annuum*) Rhizosphere

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Abstract

Heavy metals adversely affect plants, microbes and human health. Considering these, plant growth promoting rhizobacteria was isolated from metal polluted chilli rhizosphere and characterized. Indigenous strain CPSB1 identified as *Pseudomonas aeruginosa* by 16S rRNA gene sequence analysis showed higher tolerance to Ni (800:400 µg/ml), Zn (2000:800 µg/ml), Pb (1800:1000 µg/ml) and Cr (1000:400 µg/ml) on solid: liquid medium, respectively. *Pseudomonas aeruginosa* solubilized 12.3 and 13.3 µg/ml P with 400 µg/ml of Zn and Cr, respectively. Strain CPSB1 secreted IAA, siderophores, cyanide, ammonia and ACC deaminase under metal stress. Plant growth regulators gradually decreased with increasing concentrations of metals but were retained even at higher concentrations. *Pseudomonas aeruginosa* reduced Cr (VI) under aerobic conditions which completely reduced Cr (VI) after 120h at pH 8 and 35°C. Scanning electron microscopy (SEM) and Fourier Transform Infrared (FT-IR) Spectroscopy revealed cellular damage and alteration in cell surface functional groups, respectively. The metals uptake by *P. aeruginosa* CPSB1 was validated by energy dispersive X-ray (EDX) spectroscopy. The results suggest that *P. aeruginosa* endowed with plant growth promoting activities and chromium reducing ability could play a significant role in reducing chromium toxicity and supplying essential nutrients and hormones to plants, even in metal contaminated soils.

Keywords: *Pseudomonas aeruginosa*; Cellular Damage; Plant Growth Regulators; Hexavalent Chromium; Bioremediation

Abbreviations


Introduction

Industrial effluents, tanning industries, sewage wastes and many other metal discharging industries are the major sources of environmental pollution that adds considerable amount of toxic metals to the environment including soils. The heavy metals which are biologically non-degradable and persist in the environment, cause a serious threat to plants [1], soil fertility [2], microbial populations and their associated activities [3] and via food chain, human health [4]. These problems warrant urgent attention so that the health of soils and concurrently the production of crops are preserved. In this context, certain microorganisms especially belonging to plant growth promoting rhizobacteria (PGPR) group endowed with the distinctive property of heavy metal tolerance and plant growth promotion have been identified [5]. The soil microbes showing tolerance to one or multi-metals detoxify/reduce the deleterious effects of heavy metals by several mechanisms including exclusion, extracellular and intracellular sequestration of metal ions and transformation of heavy metals to less toxic forms [6,7]. In addition to metal tolerance, certain bacteria also show resistance to various antibacterial drugs (antibiotics) largely due to alterations in the genetic architecture of the organism [8]. This is due to the fact that antibiotics and heavy metal resistance genes are transferred together during conjugation [9]. The possession of dual properties i.e., the ability to tolerate higher level of metals and to display resistance against antibiotics by any PGPR makes such organism an interesting one in crop production practices.

Among the most prominent heavy metal contaminants, chromium, a product of chrome plating, wood processing, metal chelating and many other industries is one of the most hazardous metals [10] and adversely affects both plants [11] and microbes [12]. Among nine oxidation states, a highly stable Cr (VI) is more toxic to microbes [13] and plants [14] as compared to other forms of chromium. Chromium in general is inhibitory due to- (i) high wa-

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Research Article

IN VITRO ASSAY OF BIOMOLECULES, SYNTHESIS OF STRESS REDUCING PROLINE AND PERFORMANCE OF METAL TOLERANT BRADYRHIZOBIUM INOCULATED GREENGRAM (Vigna radiata L. Wilczek) UNDER METAL STRESS

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Abstract: Heavy metals are serious environmental pollutants and deleteriously affect the sustainability of microbes, plants and humans. Considering the toxicity of heavy metals, the present study was designed to isolate metal tolerant plant growth promoting rhizobacteria and to assess their plant growth promoting activities in the presence and absence of heavy metals. The best performing metal tolerant Bradyrhizobium strain C4 was selected to evaluate its impact on biological and chemical properties of greengram grown under metal stress. Bradyrhizobium sp. (vigna) strain C4 showed maximum tolerance to copper (1600 μg/ml), cadmium (200 μg/ml) and chromium (400 μg/ml) and produced siderophore, ammonia, cyanogenic compounds and synthesized indole-3-acetic acid under metal stress. Bradyrhizobium strain C4 enhanced the overall growth of greengram plants grown in soils stressed with/without varying concentrations of copper, cadmium and chromium. Proline concentration in greengram plants increased with increasing concentration of metals but declined significantly in Bradyrhizobium sp. (vigna) inoculated plants compared to control plants. The intrinsic abilities of growth promotion, enhanced performance of metal tolerant Bradyrhizobium sp. (vigna) inoculated plants and reduction in proline level of the inoculated plants grown under metal stress are indicative that Bradyrhizobium sp. (vigna) could be used for developing rhizobial inoculant for optimizing the production of greengram in soils polluted even with copper, cadmium and chromium.

Keywords: Bioremediation, Bradyrhizobium, Greengram, Heavy Metals, Proline.


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Introduction

Heavy metals discharged from industrial operations such as smelting, mining, metal forging, manufacturing of alkaline storage batteries and combustion of fossil fuel are considered a major threat to the environmental sustainability [1]. Moreover, the agricultural activities like application of agrochemicals and use of sewage in agricultural fields also adds a considerable amount of metals to the soils [2]. The discharged heavy metals persist in the environment because they cannot be degraded biologically [3]. And hence, following uptake, metals severely affect the composition and functions of microbiota [4] and plants [5] and via food chains, the animals [6] and humans [7]. Phytoxicologically, heavy metal inhibits antioxidative enzymes, impair ionic transport and redox potential of the cell and damage DNA [6,9]. Also, heavy metals adversely affect respiration process, protein synthesis and carbohydrate metabolism of plants [10]. To overcome metal stress, plants have evolved certain mechanisms. For instance, they store toxic metals in roots (bioaccumulation) and hence, prevent its translocation to other organs [11]. Also, plants secrete complex compounds that reduce metal availability in soil, exclude metal through selective uptake, immobilize and accumulate metal within vacuoles, increase production of metal-binding compounds and metal-tolerant enzymes. In this regard, amino acid for example, proline, an antioxidant and a free radical scavenger accumulates when plants are exposed to excessive stress. And hence, the accumulation of proline is considered one of the most important physiological strategies employed by higher plants to cope with toxicity under various stresses like heavy metals, salinity and drought[12]. Proline secreted by plants protects the cell membrane and enzymes [13] and also provide energy for growth and survival under stressed conditions [14]. Additionally, there are reports which suggest that proline can influence- (a) mitochondrial functions (b) cell proliferation or cell death and (iii) specific gene expression leading to protection of plants from abnormal environmental conditions. Plant growth promoting rhizobacteria (PGPR) among heterogeneously distributed soil microbiota obviate metal toxicity involving mechanisms such as biosorption, immobilization through the excretion of organic acids or bioleaching, bio-mineralization, intracellular accumulation and enzyme catalyzed transformation [4]. Consequently, after application, they improve the health of the plants in polluted soils [15] by supplying important plant nutrients like N (nitrogen fixers) and P (phosphate solubilizers), production of phytohormones and by disease suppression [16]. Considering such physiologically important traits, the use of agronomically inexpensive and vital metal tolerant PGPR has become one of the most preferred choices due in part to its easy to operate option and safety in bioremediation strategies. The present study was therefore, aimed at identifying metal tolerant PGPR, evaluating their plant growth promoting activities under metal stress and assessing the impact of metal tolerant strain on biological and chemical properties of greengram, grown under metal stressed soils.

Materials and Methods

Isolation of bacterial cultures and sensitivity/resistance to metals

Soil samples were collected from the rhizospheres of garlic (Allium sativum) and cabbage (Brassica oleracea) grown in fields receiving sewage. Soil samples were serially diluted in sterile normal saline solution and spread evenly onto nutrient
Assessment of Glyphosate and Quizalofop Mediated Toxicity to Greengram [Vigna radiata (L.) Wilczek], Stress Abatement and Growth Promotion by Herbicide Tolerant Bradyrhizobium and Pseudomonas species

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A B S T R A C T

Continued and widespread use of herbicides for controlling weeds and indirectly enhancing crop production often results in reduction in soil fertility and via food chain, human health. Realizing this threat, herbicide tolerant plant growth promoting rhizobacterial strains were isolated and identified. Of the total 40 bacterial strains, Bradyrhizobium sp. strain R5 and Pseudomonas sp. strain PS6 tolerated 3200 and 800 μg ml⁻¹ of glyphosate and Quizalofop P ethyl, respectively. Bradyrhizobium sp. strain R5 and Pseudomonas sp. strain PS6 produced indoleacetic acid, exopolysaccharides, siderophores, ammonia and solubilized inorganic P even in the presence of herbicides. Scanning electron microscopic and CLSM images revealed a clear toxicity of herbicides to bacterial cells. The toxicity of herbicides to greengram plants increased with increasing rates of glyphosate and quizalofop. The herbicide tolerant Bradyrhizobium strain R5 and Pseudomonas sp. strain PS6 when used with herbicide, considerably reduced toxicity to greengram plants. For example, Bradyrhizobium strain R5 significantly increased the nodule number, nodule dry mass, root and shoot length, root and shoot weight and total chlorophyll content by 10, 33 3, 16, 68, 23 and 6% respectively, whereas, inoculation of Pseudomonas sp. strain PS6 enhanced the measured parameters by 2, 7, 3, 12, 25, 22 and 20%, respectively, as compared to the plants grown solely with 1444 μg kg⁻¹ of glyphosate. Among the two bacterial strains, Bradyrhizobium R5 showed better results under identical herbicide stress as compared to the Pseudomonas PS6. In general, the present findings suggest that both Bradyrhizobium sp. strain R5 and Pseudomonas sp. strain PS6 could be exploited as an efficient microbial inoculant to increase the productivity of greengram while reducing the toxicity of glyphosate and quizalofop.

Keywords
Greengram, Herbicide toxicity, PGPR, Bioremediation, Growth promotion.

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Introduction

Environmental pollution due to wide spread use of agrochemicals is a major global threat to the sustainability of agro-ecosystems. Among various agrochemicals applied in agricultural practices, the intensive and uncontrolled use of herbicides over the years has caused serious problems (Ozkara et al., 2016). The productivity of legumes on the other hand often suffers from weed competition. And hence, to control weeds and consequently to enhance crop production, herbicides are applied consistently. Among legumes, greengram is known for its detoxification activities and is used to refresh
Assessment of Heavy Metals Toxicity on Plant Growth Promoting Rhizobacteria and Seedling Characteristics of *Pseudomonas putida* SFB3 Inoculated Greengram

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**Abstract**

Heavy metals present a great environmental concern, because of their adverse impact on microflora, plants and humans. The growing environmental awareness necessitates the development of effective and inexpensive methods for metal removal. In the present study, an attempt was made to examine the plant growth promoting abilities of the rhizobacteria isolated from metal contaminated fields. A total of 113 rhizobacterial isolates belonging to genera *Bacillus, Pseudomonas, Azotobacter* and *Rhizobium* were isolated from rhizospheric soils of bajra grown in the fields near Mathura road, U.P., India. The rhizobacterial isolates were characterized biochemically and evaluated for their plant growth promoting traits such as production of indole acetic acid (IAA), ammonia (NH$_3$), hydrogen cyanide (HCN), siderophore and catalase. Also, the metal tolerant ability of the bacterial cultures was determined. All isolates were positive for catalase and NH$_3$ production. All isolates of *Pseudomonas* spp., *Bacillus* spp. and *Azotobacter* spp. produced IAA whereas only 57% *Rhizobium* spp. produced IAA. Among the bacterial isolates, *Pseudomonas putida* strain SFB3 (identified using 16S rRNA gene sequence analysis, GenBank accession no. MF284668) showed high level of tolerance to multiple heavy metals and exhibited significant plant growth promoting activities even under metal stress. The strain SFB3 when used as an inoculant enhanced the germination efficiency and seedling vigour of greengram besides increasing the plumula and radicle length both in metal free and metal stress conditions. *Pseudomonas putida* strain SFB3 showing tolerance to multiple heavy metals and exhibiting PGP traits hold promise as effective PGPR for enhancing crop production when applied as biofertilizer under field conditions.

**Keywords:** Greengram; Metal Tolerance; PGPR; *Pseudomonas*

**Introduction**

Heavy metals are generally referred to as those metals which possess a specific density greater than 5 g/cm$^3$ [1]. Rapid industrialization and various anthropogenic activities have been responsible for increased heavy metal release to the environment causing negative impacts on agriculture and human health. Due to non-biodegradable and persistent nature, the excessive accumulation of heavy metals into soils becomes most dangerous to crop plants and affects structure and microbial composition of soils and their activity.

This in turn cause reduction in fertility and concurrently results in yield losses [2,3]. They also pose significant threat to human beings via the food chain [4]. According to the World Health Organization (WHO) Cd, Cr, Co, Cu, Pb, Ni, Hg and Zn are the most hazardous metals [5]. Conventional methods to remediate heavy metals contaminated site are excavation and solidification or stabilization.

Even though these technologies are suitable to contain contamination but they cannot permanently remove metals from the polluted sites [6]. In addition, these methods are expensive, and generates hazardous by-products. To circumvent such problems, biological methods have been found as inexpensive, easy to operate and they do not produce secondary pollution [7]. Among biological materials used in metal detoxification, microorganisms endowed with metal tolerance ability can be exploited to remove, concentrate and recover metals from contaminated sites [8]. In this context, studies have been conducted to assess the impact of various plant growth promoting bacteria (PGPB) for effective bio-remediation of metal contaminated soils. When used as inoculant under metal stressed environment such PGPR stimulates plant growth by: (i) supplying N [9] and P (ii) phytohormone production [10] (iii) enhancing plant resistance to metals and protection of plants from pathogens through release of volatile components (acetoin and 2, 3-butanediol) [11], synthesis of 1-aminocyclo-
Inhibition of growth and biofilm formation of clinical bacterial isolates by NiO nanoparticles synthesized from *Eucalyptus globulus* plants

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**ABSTRACT**

Nanotechnology based therapeutics has emerged as a promising approach for augmenting the activity of existing antimicrobials due to the unique physical and chemical properties of nanoparticles (NPs). Nickel oxide nanoparticles (NiO-NPs) have been suggested as prospective antibacterial and antitumor agent. In this study, NiO-NPs have been synthesized by a green approach using *Eucalyptus globulus* leaf extract and assessed for their bactericidal activity. The morphology and purity of synthesized NiO-NPs determined through various spectroscopic techniques like UV-Visible, FT-IR, XRD, EDX and electron microscopy differed considerably. The synthesized NiO-NPs were pleomorphic varying in size between 10 and 20 nm. The XRD analysis revealed the average size of NiO-NPs as 19 nm. The UV-Vis spectroscopic data showed a strong SPR of NiO-NPs with a characteristic spectral peak at 396 nm. The FTIR data revealed various functional moieties like C=C, C=N, C=H and O=H which elucidate the role of leaf biomolecules in capping and dispersal of NiO-NPs. The bioactivity assay revealed the antibacterial and anti-biofilm activity of NiO-NPs against ESBL (+) *E. coli*, *P. aeruginosa*, methicillin sensitive and resistant *S. aureus*. Growth inhibition assay demonstrated time and NiO-NPs concentration dependent decrease in the viability of treated cells. NiO-NPs induced biofilm inhibition was revealed by a sharp increase in characteristic red fluorescence of PI, while SEM images of NiO-NPs treated cells were irregular shrink and distorted with obvious depressions/indentations. The results suggested significant antibacterial and anti-biofilm activity of NiO-NPs which may play an important role in the management of infectious diseases affecting human health.

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1. Introduction

The fabrication of nano-scale (<100 nm) particles and their subsequent applications in industrial and biomedical area are increasing consistently due to their larger catalytic property and higher surface area. Recently, the interest in synthesizing nanoparticles has considerably been increased due to their practical applications in many important fields such as memory storage devices [1], sensors [2,3], magnetic resonance imaging [4], photocatalytic [5], drug delivery [6,7], catalysis [8], and in the treatment of infectious diseases [9]. Among various nanomaterials available, NiO nanoparticles (NiO-NPs) have attracted greater attention due to their flexible properties such as electron transfer capability, super-capacitance properties, electro catalysis, and high chemical stability [10,11]. Due to these, many physical and chemical methods have been used successfully to synthesize NiO-NPs [12–14]. For instance, NiO nanoparticles films having high surface area have been produced employing techniques like laser liquid ablation, electrodeposition, spin coating, sole-gel, chemical bath deposition, and spray pyrolysis [15–18]. Most of these methods however, use toxic chemicals, organic solvents, and non-biodegradable materials as reducing and stabilizing agents which may cause environmental problems [19]. To counteract these problems, there is urgent need to find inexpensive and environmentally friendly nanomaterials. In this regard, the green synthesis of metallic nanoparticles using plant extracts offer an attractive and inexpensive alternative to conventional physical and chemical methods [20]. Bioactive
Mitochondrial and Chromosomal Damage Induced by Oxidative Stress in Zn$^{2+}$ Ions, ZnO-Bulk and ZnO-NPs treated Allium cepa roots

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Large-scale synthesis and release of nanomaterials in environment is a growing concern for human health and ecosystem. Therefore, we have investigated the cytotoxic and genotoxic potential of zinc oxide nanoparticles (ZnO-NPs), zinc oxide bulk (ZnO-Bulk), and zinc ions (Zn$^{2+}$) in treated roots of Allium cepa, under hydroponic conditions. ZnO-NPs were characterized by UV-visible, XRD, FT-IR spectroscopy and TEM analyses. Bulbs of A. cepa exposed to ZnO-NPs (25.5 nm) for 12 h exhibited significant decrease (23 ± 8.7%) in % mitotic index and increase in chromosomal aberrations (18 ± 7.6%), in a dose-dependent manner. Transmission electron microcopy and FT-IR data suggested surface attachment, internalization and biomolecular intervention of ZnO-NPs in root cells, respectively. The levels of TBARS and antioxidant enzymes were found to be significantly greater in treated root cells vis-à-vis untreated control. Furthermore, dose-dependent increase in ROS production and alterations in $\Delta\psi_m$ were observed in treated roots. FT-IR analysis of root tissues demonstrated symmetric and asymmetric P=O stretching of $>$PO$_2^-$ at 1240 cm$^{-1}$ and stretching of C-O ribose at 1060 cm$^{-1}$, suggestive of nuclear damage. Overall, the results elucidated A. cepa, as a good model for assessment of cytotoxicity and oxidative DNA damage with ZnO-NPs and Zn$^{2+}$ in plants.

ZnO nanomaterials have enormous applications in various fields, such as manufacturing of solar cells, sensors$^{1,2}$, piezoelectric devices, light emitting diodes, semiconductors$^3$, and paints$^4$. Besides, they are widely used in health applications such as, sun screens, cosmetics, bio-imaging, diseases diagnostics, and cancer treatment$^5$–$^8$. The rationale for their extensive use is the unique and fascinating electrical, optical and mechanical properties, including absorption of solar energy, high catalytic efficiency, high photo sensitivity, stability, and high band gap ($3.37$ eV) with large exciton binding energy ($60$ MeV)$^9$–$^{13}$. Furthermore, ZnO-NPs are reported to inhibit bacterial growth due to release of zinc ions from ZnO-NPs$^{14}$, and manifest toxicity via generation of reactive oxygen species (ROS), such as hydrogen peroxide$^{15}$. Increased expression of general stress response gene ($dnaK$) and oxidative stress genes ($ahpC$ and $katA$) following exposure to ZnO-NPs, suggested induced oxidative stress in bacteria$^{16}$. Moreover, ZnO-NPs induced oxidative stress and apoptosis have been reported in HepG2 and MCF-7 cells$^{17}$, and DNA damage in human lymphocytes$^{18}$.

Lately, phytotoxicity assessment of ZnO-NPs has been envisaged as a subject of investigation, since plants at the first trophic level offer a large surface area to NPs in their habitats such as soil, air, and water$^{19}$. Primarily, root is the region of plant system, where NPs encounter first contact, starting from adsorption at root tips, rhizodermis, lateral root junctions and other plant surfaces viz. cuticle, bark, hydathodes, and stigma$^{19}$. Uptake of NPs may occur through micro- and nanometer in size orifices in plants, and subsequently translocated or aggregated at the cellular surfaces, plasmodesmata, and/or inside the cell$^{19}$. Accumulation and long term persistence

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Ensifer adhaerens for heavy metal bioaccumulation, biosorption, and phosphate solubilization under metal stress condition

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ABSTRACT

In this study, a bacterial strain was recovered from the chickpea root surface and its heavy metal tolerance ability, bioaccumulation, biosorption, chromium reduction potential, and phosphate solubilization activity were determined. Bacterial strain (OS3) was molecularly characterized through 16S rRNA partial gene sequencing and confirmed strain sequences belonged to the Ensifer adhaerens, invetigate by the BLASTn and phylogenetic analysis and achieved accession number from Genbank: HE681418.1. Strain OS3 was able to tolerate up to 250 μg/ml Cd, 500 μg/ml Cr; 800 μg/ml each of Cu and Zn and 1000 μg/ml Ni. Moreover, strain OS3 was able to bioaccumulate and biosorb varying concentrations of heavy metals and showed maximum accumulation of Ni (95%) and least accumulation of Pb (74%) at a temperature of 34 °C and pH 7. The Langmuir and Freundlich adsorption constants were evaluated from the isotherms with a correlation coefficient (r² > 0.98). The strain reduced 200 μg/ml of Cr (VI) completely after 96 h growth at 35 °C. Apart from bioremediation ability, bacterial strain OS3 expressed a significant inorganic phosphate (P) solubilizing activity and solubilized inorganic P upto 303 μg/ml with a 2.2 solubilization index. Also, strain OS3 demonstrated plant growth promoting traits even under metal stress and secreted IAA, siderophore, HCN and ammonia under in vitro conditions. This study demonstrated that E. adhaerens survived well, even when exposed to higher concentrations of heavy metals and retained plant growth promoting activities. The biosorption ability, chromium reducing potential and ability to secrete plant growth regulators makes this organisms an interesting choice for metal removal (bioremediation) and enhancing crop production even in metal polluted soils.

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1. Introduction

The modern era, with its increasing technological advancement, rapid industrialization and subsequent huge waste generation have brought alarming detrimental effects on the natural ecosystem. A large number of industries, mining and other anthropogenic activities are discharging heavy metals and causing severe organic pollution [1]. Through natural processes these heavy metals and organic compounds mitigate in soil system by several intricate pathways and whenever the concentration of these pollutants exceeds the threshold limit, then pollute the soil and consequently the soil becomes barren or infertile [2]. Soil is one of the most important habitat that harbors diverse micro-flora and fauna which are affected deleteriously by heavy metal pollution [3]. However, huge amounts of organic or metallic pollutants can not easily be removed from the soil environment. Most of the toxic metals were also recognized as a major inhibitor of biodegradation of organic pollutants [4]. These heavy metals after uptake, accumulate in living organisms and create physiological and metabolic disorders [5]. The toxic effects of heavy metals result mainly from the interaction of metals with enzymes and blocks the biological reaction of metabolic pathways [6]. Each heavy metal has unique biological functions and toxicities. For example, copper and zinc acts as a cofactor for several enzymes and enhance the microbial growth at low concentrations, but repress growth at high concentrations while nickel, cadmium and lead have high toxicity even at low concentrations [7]. The uncontrolled discharge of heavy metals from various industries, cause environmental and human health problems and also increases the cost of wastewater treatment significantly [8]. Since ages, number of methods for example: ion exchange, precipitation, electrochemical treatments, membrane filtration technology, activated charcoal and carbon for adsorption and several other chromatographic techniques have been

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Titanium dioxide nanoparticles preferentially bind in subdomains IB, IIA of HSA and minor groove of DNA

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Titanium dioxide nanoparticles (TiO₂-NPs) interaction with human serum albumin (HSA) and DNA was studied by UV–visible spectroscopy, spectrofluorescence, circular dichroism (CD), and transmission electron microscopy (TEM) to analyze the binding parameters and protein corona formation. TEM revealed protein corona formation on TiO₂-NPs surface due to adsorption of HSA. Intrinsic fluorescence quenching data suggested significant binding of TiO₂-NPs (avg. size 14.0 nm) with HSA. The Stern–Volmer constant \(K_v\) was determined to be \(7.6 \times 10^{2} \text{ M}^{-1}\) \(\left( r^2 = 0.98 \right)\), whereas the binding constant \(K_b\) and number of binding sites \(n\) were assessed to be \(5.82 \times 10^{10} \text{ M}^{-1}\) and 0.97, respectively. Synchronous fluorescence revealed an apparent decrease in fluorescence intensity with a red shift of 2 nm at \(\Delta \lambda = 15 \text{ nm}\) and \(\Delta \lambda = 60 \text{ nm}\). UV–visible analysis also provided the binding constant values also provided the binding constant values for TiO₂-NPs-HSA and TiO₂-NPs-DNA complexes as \(2.8 \times 10^{4} \text{ M}^{-1}\) and \(5.4 \times 10^{3} \text{ M}^{-1}\). The CD data demonstrated loss in \(\alpha\)-helicity of HSA and transformation into \(\beta\)-sheet, suggesting structural alterations by TiO₂-NPs. The docking analysis of TiO₂-NPs with HSA revealed its preferential binding with aromatic and non-aromatic amino acids in subdomain IIA and IB hydrophobic cavity of HSA. Also, the TiO₂-NPs docking revealed the selective binding with A-T bases in minor groove of DNA.

**Keywords:** Human serum albumin; fluorescence quenching; TiO₂-NPs; circular dichroism; docking

1. Introduction

Nanoparticles (NPs) due to their unique properties have attracted significant interest in their applications in diverse areas including biomedical sciences as nano-vaccines and nano-drugs (Saptarshi, Duschl, & Lopata, 2013). However, our knowledge about the bio-compatibility and risks of exposure to nanomaterials is limited. In a biological system, NPs may interact with biomolecules viz. proteins, nucleic acids, lipids, and biological metabolites, due to their smaller size and large surface area (Saptarshi et al., 2013). Indeed, adsorption of proteins on the NPs surface is of particular importance. As soon as, the NPs are in contact with biological system (tissues, cells or blood) the surface of NPs gets covered by a thin layer of soluble proteins forming a protein corona. In general, the NPs–protein corona can influence the biological reactivity of the NPs (Casals, Pfaller, Duschl, Oostingh, & Puntes, 2010; Cedervall et al., 2007). Understanding of NPs–protein interactions could stimulate surface modifications of NPs to adsorb functional proteins or drug molecules for in vivo delivery, which has nano-toxicological implications and safety concerns. Hence, it is imperative to understand the molecular mechanisms of NPs interaction with biological system. Proteins such as albumin, fibrinogen, gamma globulins, complement factors, and 1-antitrypsin are the constituents of human plasma, which represents about 50% of the total blood volume (Aggarwal, Hall, McLeland, Dobrovolskaia, & McNeil, 2009). Human serum albumin (HSA) being the most abundant protein is present at a concentration of \(\sim 0.6 \text{ mM}\) in plasma (Quinlan, Martin, & Evans, 2005). It is a globular protein consisting of 585 amino acids including 18 tyrosines, 6 methionines, 1 tryptophan (Trp 214), 17 disulfide bridges, and only one free thiol (Cys 34) moiety. Several exogenous and endogenous ligands have been reported to bind either at Sudlow site I (located at sub-domain IIA) or at site II (located at sub-domain IIIA) of HSA (Quinlan et al., 2005). Binding of ligands with active sites of proteins can change their structure and function, which may cause toxic effects (Lundqvist et al., 2008). Lately, there are several reports on interaction of NPs with proteins and corona formation on particles surface, which varies depending on the type of NPs, as well as

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p53, MAPKAPK-2 and caspases regulate nickel oxide nanoparticles induce cell death and cytogenetic anomalies in rats

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1. Introduction

NiO-NPs are increasingly used in ceramic material, catalysts, battery electrode, electronic component, biosensors, electrochromic films, magnetic materials and diesel-fuel additive [1, 2]. The direct aerial emission of NiO-NPs generated from welding fumes during the coastal region developments have the tendency to pollute surface waters through leakages, spills and indirect stormwater runoff from land [3]. The utilization of Ni-NPs to catalyze the reversible hydration of CO2 to carbonic acid, led to its utilization to point flue sources like air-conditions outlets and power plants [4, 5]. Additionally, NiSO4, NiO, Ni(OH)2 and crystalline Ni are well known as an environmental pollutant and classified as Group 1 carcinogenic agents to humans by IARC [6, 7].

Most of the in vivo studies on NiO-NPs were directed towards its pulmonary pathology. Rats intratracheally instilled with NiO-NPs (<10 nm) exhibited increase in the bronchiolar alveolar lavage fluid (BALF), enhancement in the proinflammatory mediators (IL-1β, IFN-γ, MIP-2) and histological alterations [8]. NiO-NPs (500 cm2/ml) exposure for 24 h increased the polymorphonuclear neutrophils in BALF of rats [9]. Male rats exposed to NiO-NPs (20 nm) for 4 weeks in nebulizer chamber exhibited inflammatory responses and biopersistence of NPs in lungs [10]. NiO-NPs (26 nm) intratracheally instilled in rats for 6 months enhanced the neutrophil, macrophages and cytokine-induced neutrophil chemotactant 1 (CINC-1) concentrations in BALF, including an extensive vacuolization in the cytoplasm of alveolar macrophages [11]. Recently, male rats exhibited neutrophilic and eosinophilic inflammation after 4 days of post instillation of NiO-NPs, involving the release of intracellular eotaxin by the accumulation of solubilized Ni²⁺, which also caused cellular disintegration [12]. In a pharyngeal aspiration study, NiO-NPs exposed female rats exhibited increase in polymorphonuclear leukocytes, lymphocytes, proinflammatory cytokines,
Copper doping enhanced the oxidative stress–mediated cytotoxicity of TiO\textsubscript{2} nanoparticles in A549 cells

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Abstract
Physicochemical properties of titanium dioxide nanoparticles (TiO\textsubscript{2} NPs) can be tuned by doping with metals or nonmetals. Copper (Cu) doping improved the photocatalytic behavior of TiO\textsubscript{2} NPs that can be applied in various fields such as environmental remediation and nanomedicine. However, interaction of Cu-doped TiO\textsubscript{2} NPs with human cells is scarce. This study was designed to explore the role of Cu doping in cytotoxic response of TiO\textsubscript{2} NPs in human lung epithelial (A549) cells. Characterization data demonstrated the presence of both TiO\textsubscript{2} and Cu in Cu-doped TiO\textsubscript{2} NPs with high-quality lattice fringes without any distortion. The size of Cu-doped TiO\textsubscript{2} NPs (24 nm) was lower than pure TiO\textsubscript{2} NPs (30 nm). Biological results showed that both pure and Cu-doped TiO\textsubscript{2} NPs induced cytotoxicity and oxidative stress in a dose-dependent manner. Low mitochondrial membrane potential and higher caspase-3 enzyme (apoptotic markers) activity were also observed in A549 cells exposed to pure and Cu-doped TiO\textsubscript{2} NPs. We further observed that cytotoxicity caused by Cu-doped TiO\textsubscript{2} NPs was higher than pure TiO\textsubscript{2} NPs. Moreover, antioxidant N-acetyl cysteine effectively prevented the reactive oxygen species generation, glutathione depletion, and cell viability reduction caused by Cu-doped TiO\textsubscript{2} NPs. This is the first report showing that Cu-doped TiO\textsubscript{2} NPs induced cytotoxicity and oxidative stress in A549 cells. This study warranted further research to explore the role of Cu doping in toxicity mechanisms of TiO\textsubscript{2} NPs.

Keywords
Doping, Cu-doped TiO\textsubscript{2} nanoparticles, cytotoxicity, oxidative stress, A549 cells

Introduction
Nanotechnology provides opportunity to develop nanoparticles (NPs) with unique features that have potential to be applied in several fields. The NPs are defined as materials, structures, or devices that have at least one dimension in the range of 1–100 nm. The NPs are being utilized in every aspect of human life including medical science, agriculture, and defense industries.\textsuperscript{1,2} However, the intentional\textsuperscript{3,4} or unintentional\textsuperscript{5} exposure of these NPs may pose health hazard to the environment and human.\textsuperscript{6–9} Being smaller than cells and organelles, NPs can penetrate basic biological structures that may, in turn, disrupt their function.\textsuperscript{10,11}

Titanium dioxide (TiO\textsubscript{2}) NPs are now being produced in a large industrial scale and used widely due
MWCNTs functionalization and immobilization with anti-Brucella antibody; towards the development of a nanosensor

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Abstract

Brucellosis is endemic worldwide due to the nomadic heritage of drinking fresh milk from goats, sheeps and camels. This study aims at developing an antibody based sensor for the detection of Brucella, wherein the binding of Brucella to the sensor will be detected by measuring the changes in electrical signals via cyclic voltammetric properties. The morphology of the used MWCNTs was observed under SEM, FESEM, and TEM. The MWCNTs (ropes like structure) used for the development of sensor were 2–4 μm in length and ~10–15 nm in diameter as observed under SEM, FESEM, and TEM. MWCNTs were functionalized via acid treatment/oxidation process and the functional behavior of molecules was confirmed with FTIR spectroscopy. Antibodies were conjugated onto the functionalized MWCNTs using classical EDC coupling reactions. When observed under SEM, binding of Brucella abortus to these anti-Brucella antibody functionalized MWCNTs was clearly observed. Finally, these antibody functionalized MWCNTs were deposited on aluminum sheets for the voltammetric detection of Brucella. Results indicate that the antibody functionalized MWCNTs prepared in this study can be used successfully for the detection of Brucella.

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1. Introduction

Brucellosis is a chronic infectious zoonotic disease worldwide, which is characterized by infectious abortion and sterility in various domestic animal species and potentially debilitating infection in humans [1–4]. Brucellosis is caused by the facultative intracellular Gram-negative bacteria of the genus Brucella [1,5,6]. There are ten species of Brucella based on preferential host specificity, with three species, Brucella melitensis (mainly infecting goats and sheeps), Brucella abortus (cattle) and Brucella suis (swine) highly pathogenic to humans [7,8]. Although some developed countries in Northern Europe, Australia, USA, and Canada, have eradicated Brucellosis from livestock, the disease remains endemic in many regions of the world including Latin America, Middle East, Africa, Asia, and the Mediterranean basin [2]. Brucellosis can result in great economic losses, particularly in the food animal production sector [9]. Human Brucellosis occurs when humans come in direct contact with fluid discharges from an infected animal or through the consumption of unpasteurized dairy products [2,10]. Thus Brucellosis is a significant public health issue and a significant economic concern. Up to date, quarantine, slaughter and vaccination are mainstay arsenals for the control of Brucellosis [11,12]. Towards this concern, report published by Christopher et al. [13] describe the detail of pathogenicity and laboratory diagnosis of Brucellosis whereas others displayed the influences of Brucellosis in humans [14–16].

For an effective control of the disease it is important to accurately detect the pathogen in affected subjects and in the contaminated milk one of the primary source of pathogen. Various techniques are being used for the detection of Brucella including Rose Bengal plate agglutination test (RBPT), microbial culture, standard tube agglutination test (SAT), Coombs test and ELISA [17–21]. These techniques require trained manpower, costly...
Photocatalytic TMO-NMs adsorbent: Temperature-Time dependent Safranine degradation, sorption study validated under optimized effective equilibrium models parameter with standardized statistical analysis

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In this paper, chemically synthesized copper oxide nanoparticles (CuO-NPs), were employed for two processes: one is photocatalytic degradation and second one adsorption for the sorption of safranine (SA) dye in an aqueous medium at pH = 12.01. The optimized analytes amount (nano-adsorbent = 0.10 g, conc. range of SA dye 56.13 ppm to 154.37 ppm, pH = 12.01, temperature 303 K) reached to equilibrium point in 80 min, which acquired for chemical adsorption-degradation reactions. The degraded SA dye data’s recorded by UV-visible spectroscopy for the occurrence of TMO-NMs of CuO-NPs at anticipated period of interval. The feasible performance of CuO-NPs was admirable, shows good adsorption capacity \( q_m = 53.676 \, \text{mg g}^{-1} \) and most convenient to best fitted results establish by linear regression equation, corresponded for selected kinetic model (pseudo second order \( R^2 = 0.9981 \), equilibrium isotherm models (Freundlich, Langmuir, Dubinin-Radushkevich (D-R), Temkin, H-J and Halsey), and thermodynamic parameters \( \Delta H^o = 75461.909 \, \text{J mol}^{-1} \), \( \Delta S^o = 253.761 \, \text{J mol}^{-1} \), \( \Delta G^o = -1427.93 \, \text{J mol}^{-1} \), \( E_a = 185.142 \, \text{J mol}^{-1} \)) with error analysis. The statistical study revealed that CuO-NPs was an effective adsorbent certified photocatalytic efficiency \( \eta = 84.88\% \) for degradation of SA dye, exhibited more feasibility and good affinity toward adsorbate, the sorption capacity increases with increased temperature at equilibrium point.

Nanomaterials (NMs) are valuable constructive material in multidisciplinary science and have various applications due to their exclusive properties such as small size, larger surface area with volume, higher distortion of surface lattice energy and high thermal reactivity etc, which illustrate beneficial requirement for numerous purposes in a manner such as adsorption, catalysis, energy conversion storage, optoelectronics and drug delivery\textsuperscript{1-5}. The organic dyes are the bigger macromolecules with different color, widely used in various industries for instance in textile, food, leather, cosmetic, plastic, leather etc. The average production of synthetic dyes from the industries is ~7 million tons worldwide. The removal of dyes in a smaller fragment molecule is a big issue for the

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Nigella sativa seed oil suppresses cell proliferation and induces ROS dependent mitochondrial apoptosis through p53 pathway in hepatocellular carcinoma cells

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ABSTRACT

Cancer is one of the life-threatening diseases and a leading cause of death worldwide. Herbal medicine has a potential of treating many diseases. Nigella sativa L. is a widely used plant in traditional systems of medicine. The cytotoxic potential of Nigella sativa seed oil (NSO) was assessed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), neutral red uptake (NRU) assays and morphological alterations in HepG2, MCF-7, A-549 and HEK293 cell lines. Further, the influence of cytotoxic concentrations (50–250 μg/ml) of NSO on oxidative stress markers (GSH and LPO), reactive oxygen species (ROS) generation, mitochondrial membrane potential (MMP) and mRNA expression of apoptotic marker genes (p53, caspase-3, caspase-9, Bax, Bcl-2) were studied. The results exhibited significant decrease in the percentage cell viability of HepG2, MCF-7 and A-549 cells in a concentration-dependent manner. However, NSO showed higher cytotoxic response in HepG2 cells and less in HEK293 cells. Therefore, HepG2 cells were selected to further investigate the underlying mechanism(s) responsible for the cytotoxic response. NSO was found to induce oxidative stress in a concentration-dependent manner, which was indicated by induction of ROS and LPO along with decrease in reduction of GSH and MMP. Quantitative real-time PCR data showed that following the exposure of HepG2 cells to NSO, the level of mRNA expression of apoptotic marker genes (p53, caspase-3, caspase-9 and bax) was up-regulated whereas, anti-apoptotic gene bcl-2 was down-regulated. The results demonstrated that NSO induced cytotoxicity and apoptosis in HepG2 cells via ROS generation, which is likely mediated through the p53 pathway.

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1. Introduction

Although there has been rapid advancement in biomedicine and associate domains, cancer remains a leading and growing cause of suffering and loss of life throughout the world (Stewart and Wild, 2014). Moreover, the incidence of many cancers, lung cancer, breast cancer, and liver cancer were the most common sites of cancer diagnosed in 2012 among men and women (Siegel et al., 2012). Cancer is differentiated by irregular growth of cell, which begin from a small number of inheritance or environmentally mutated genes (Renan, 1993). Each type of cancers is in need of a precise course of treatment that includes one or more modalities such as surgery, radiotherapy, and/or chemotherapy (Galaal et al., 2013; Shylasree et al., 2013). Even though there are limitations, but cancer patients are being successfully treated by the surgery, radiation therapy and chemotherapy (Espinosa et al., 2003; Hoskin and Ramamoorthy, 2008; Liu, 2009). Although the desired goal of chemotherapy is to get rid of the tumor cells, diverse ranges of normal cell types are also affected, leading to many adverse side effects in multiple organ systems (Nicolson, 2005; Ahles and Saykin, 2007; Zhou et al., 2007; Constantinou et al., 2008; Han et al., 2008; Howes, 2009). Such kind of debilitating effects is a main clinical problem (Johnstone et al., 2002), whereas the toxicity often limits the usefulness of anticancer agents (Johnstone et al., 2002; Kovacic, 2007). Thus, there is a critical requirement to search for anti-cancer drugs with higher efficacy, less toxicity and at an affordable cost (Fadeyi et al., 2013). Natural products have been considered as a valuable source for the anticancer drug discovery (Cragg and Newman, 2005; Svejda et al., 2010; Khan et al., 2011; Randhawa and Alghamdi, 2011; Sharma et al., 2011; Al-Oqail et al., 2013; Farshori et al., 2013; Thoppil et al., 2013; Al-Sheddi et al., 2014).

Nigella sativa L. (N. sativa), an annual herb that belongs to the Ranunculaceae family, is used as an important nutritional flavoring agent cultivated in many countries of the world in Southern Europe,
Functionalization of anti-Brucella antibody on ZnO-NPs and their deposition on aluminum sheet towards developing a sensor for the detection of Brucella

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A B S T R A C T
Brucellosis is an endemic zoonotic disease in worldwide, particularly prevalent in Central Najed region of Saudi Arabia, where the population has a nomadic heritage of drinking fresh milk from goats, sheep and camels. The present study aims to develop a simple antibody-functionalized nanomaterial-based biosensor for the detection of Brucella in milk samples. The detection of Brucella by the proposed sensor is based on measuring changes in electrical signals and study their properties. The zinc oxide nano particles (ZnO-NPs) were synthesized through solution process in liquid medium at reduced refluxing temperature. The crystalline properties of obtained ZnO-NPs were analyzed via X-ray diffraction pattern (XRD), whereas the morphology was observed with field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). The investigation reveals that obtained NPs were almost spherical in shape with an average diameter of ~10–15 nm. The binding of the anti-Brucella antibody to ZnO-NPs was verified by FTIR. Our study showed that the anti-Brucella antibody was successfully functionalized on ZnO-NPs and these functionalized NPs were also effectively deposited on aluminum sheets (Al-sheet). Finally, Al-sheets developed above were used as an electrode component for voltammetric detection via cyclic voltammetry (CV).

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1. Introduction

Brucellosis a global zoonotic disease [1–4] is a matter of special concern to Saudi Arabia, where the incidence in some region is as high as 8–8.8% [5,6]. According to some estimates more than 8000 cases/year are reported to public health authorities [7]. The incidence is particularly higher in rural areas due to the nomadic tradition of drinking raw milk [8]. Surprisingly, very few systematic reports on Brucellosis are available from the Kingdom of Saudi Arabia, central Asia, and Sub-Saharan Africa [1,9,10]. Although the reports on human Brucellosis from different regions of Saudi Arabia are available, whereas almost no report on camel Brucellosis, or on the presence of Brucella in Camel milk one of the primary sources of the disease are available [6,11]. Therefore, it is both urgent and necessary to monitor the disease in the region, especially by controlling the source of pathogen.

Devising improved methods for easy and accurate detection of Brucella in camel milk samples is crucial for controlling the disease in the region. Various immunological techniques including Rose Bengal plate agglutination test (RBPT), standard tube agglutination test (SAT), Coombs test, PCR and ELISA are being used for the detection of Brucella [12–15]. However, these techniques need costly instrumentation and skilled manpower. Therefore, there is a clear need for effective, economic and easy to use alternatives. Nanobased sensors are emerging as versatile sensors for various biological applications including the detection of pathogens [13–15]. These sensors employ various metal oxides nanostructures, having a characteristic high surface area and can function over a wider range of temperatures, voltages, and solvent conditions. Among immeasurable kinds of nanostructures such as rods, flowers, tubes, ribbons, belts, bridges, nails, spikes, whiskers,
Zinc Oxide Nanoparticles: Mechanism(s) of Cell Death Induced in Human Epidermoid Larynx Cell Line (HEp-2)

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Zinc oxide nanoparticles (ZnO-NPs, 12 ± 3 nm) have attracted due to their unique properties and immense potential application. Over various physicochemical applications, the cytotoxic profile of ZnO-NPs in human epidermoid larynx cell line (HEp-2) has not been completely determined till date. Since, human exposure to ZnO-NPs may occur through the exposure routes of inhalation and ingestion at occupational level. The HEp-2 cells were exposed to ZnO-NPs at 2-20 μg/mL concentrations for 24 h. ZnO-NPs depicted a concentration dependent cytotoxic, ROS and mitochondrial membrane potential (MMP) response observed in HEp-2 cells. The events of cytotoxicity and ROS were found to be associated with up-regulation in mRNA expressions of pro-apoptotic marker genes viz., p53, Bax, caspase-3, and caspase-9 and down-regulation of anti-apoptotic marker gene, Bcl-2. The findings of this study indicates that ZnO-NPs induced cytotoxicity in HEp-2 cells is likely through ROS generation and mitochondria mediated apoptosis involving p53, Bax/Bcl-2 and caspase-3/9 genes.

Keywords: HEP-2 CELLS; MITOCHONDRIAL MEMBRANE POTENTIAL (MMP); NANOPARTICLES; ROS GENERATION

Document Type: Short Communication

Publication date: April 1, 2017

More about this publication?
Thymol and carvacrol induce autolysis, stress, growth inhibition and reduce the biofilm formation by *Streptococcus mutans*

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**Abstract**

Organic compounds from plants are an attractive alternative to conventional antimicrobial agents. Therefore, two compounds namely M-1 and M-2 were purified from *Origanum vulgare* L. and were identified as carvacrol and thymol, respectively. Antimicrobial and antibiofilm activities of these compounds along with chlorhexidine digluconate using various assays was determined against dental caries causing bacteria *Streptococcus mutans*. The IC50 values of carvacrol (M-1) and thymol (M-2) against *S. mutans* were 65 and 54 µg/ml, respectively. Live and dead staining and the MTT assays reveal that a concentration of 100 µg/ml of these compounds reduced the viability and the metabolic activity of *S. mutans* by more than 50%. Biofilm formation on the surface of polystyrene plates was significantly reduced by M-1 and M-2 at 100 µg/ml as observed under scanning electron microscope and by colorimetric assay. These results were in agreement with RT-PCR studies. Wherein exposure to 25 µg/ml of M-1 and M-2 showed a 2.2 and 2.4-fold increase in Autolysin gene (*AtlE*) expression level, respectively. While an increase of 1.3 and 1.4 fold was observed in the super oxide dismutase gene (*sodA*) activity with the same concentrations of M-1 and M-2, respectively. An increase in the *ymcA* gene and a decrease in the *gtfB* gene expression levels was observed following the treatment with M-1 and M-2. These results strongly suggest that carvacrol and thymol isolated from *O. vulgare* L. exhibit good bactericidal and antibiofilm activity against *S. mutans* and can be used as a green alternative to control dental caries.

**Keywords:** Thymol, Carvacrol, Oral hygiene, Alternative antimicrobials, *S. mutans*

**Introduction**

The oral cavity is a complex microbial environment hosting around 600 different bacterial species and many of these bacteria are now being associated with oral diseases (Dewhirst et al. 2010; Moore and Moore 1994; Wade 2013). Dental diseases incur substantial economic losses globally accounting to 298 billion US dollar per year which is 4.6% of the total global health expenditure (Listl et al. 2015). The evidence is also now available that the recurring infections of these oral microorganisms result in a number of systemic diseases further adding to the economic losses and loss of life (Khan et al. 2015; Li et al. 2000). One of the most important etiological agents of dental caries is *Streptococcus mutans* (Loesche 1986). In addition to being classically associated with dental caries, this bacterium also causes other systemic diseases such as ulcerative colitis, endocarditis and septicemia (Kojima et al. 2012; Nobbs 2016; Robbins et al. 1977; Tunkel and Sepkowitz 2002). The biofilm formation by *S. mutans* and its ability to promote the biofilm formation by other oral bacteria also makes it more difficult to treat the infections of *S. mutans* (Ahn et al. 2008; Klein et al. 2015; Krzyściak et al. 2014). Therefore, for a good oral hygiene, it is very important to control the growth of *S. mutans* in the oral cavity. Furthermore, the unwarranted and overuse of the antibiotics in dentistry has resulted in the drug resistance among commensal as well as pathogenic bacteria of the oral cavity including *S. mutans* (Leistevuo...
SYNTHESIS AND CHARACTERIZATION OF SOME ABUNDANT NANOPARTICLES, THEIR ANTIMICROBIAL AND ENZYME INHIBITION ACTIVITY

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Although the antimicrobial activity of the engineered nanoparticles (NPs) is well known, the biochemical mechanisms underlying this activity are not clearly understood. Therefore, four NPs with the highest global production, namely SiO2, TiO2, ZnO, and Ag, were synthesized and characterized. The synthesized SiO2, TiO2, ZnO, and Ag NPs exhibit an average size of 11.12, 13.4, 35, and 50 nm, respectively. The antimicrobial activity of the synthesized NPs against bacteria and fungi were also determined. NPs-mediated inhibition of two very important enzymes, namely urease and DNA polymerase, is also reported. The synthesized NPs especially Ag and ZnO show significant antimicrobial activity against bacteria and fungi including methicillin-resistant Staphylococcus aureus even at low concentration. The DNA polymerase activity was inhibited at a very low concentration range of 2–4 μg/ml, whereas the urease activity was inhibited at a high concentration range of 50–100 μg/ml. Based on their ability to inhibit the urease and DNA polymerase, NPs can be arranged in the following order: Ag > ZnO > SiO2 > TiO2 and Ag > SiO2 > ZnO > TiO2, respectively. As the synthesized NPs inhibit bacterial growth and suppress the activity of urease and DNA polymerase, the use of these NPs to control pathogens is proposed.

Keywords: nanoparticles, antimicrobial activity, urease, DNA polymerase

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Evaluation of cytotoxic responses of raw and functionalized multi-walled carbon nanotubes in human breast cancer (MCF-7) cells

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A B S T R A C T
The present investigation was aimed to discover the activity of raw (RCNTs) and functionalized multi-walled carbon nanotubes (FCNTs) for their cytotoxic potential in human breast cancer (MCF-7) cells. The RCNTs were functionalized with the acid treatment method and were characterized by SEM and TEM. The morphology of RCNTs and FCNTs were found to be variable with a size range of ~10–15 nm diameter were as the length goes up to 2–4 μm. For, cytotoxicity assessments, MCF-7 cells were exposed to RCNTs and FCNTs at 5–400 μg/ml concentrations for 24 h. The cytotoxicity was measured by MTT and NRU assays, and cellular morphology using phase contrast microscope. Further, intracellular reactive oxygen species (ROS) generation and mitochondrial membrane potential (MMP) were also studied. The results of MTT and NRU assays exhibited a concentration-dependent decrease in cell viability of MCF-7 cells. The RCNTs and FCNTs exposed cells were also found to alter the normal morphology of MCF-7 cells. Furthermore, the cells showed significant induction in ROS generation and reduction in MMP level. The results of this study, demonstrated that RCNTs and FCNTs has the potential to induce cytotoxicity in MCF-7 cells which could be mediated through the reactive oxygen species (ROS) generation.

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1. Introduction
Nanotechnology, particularly the development of nanoparticles and manipulation of materials at nanometer scale has emerged the new global focus for a wide spectrum of basic sciences and applied engineering [1]. Various types of nano-materials have novel biological and chemical properties and most of them are not naturally occurring [2–5]. Nanomaterials possessing novel physical, chemical and functional properties have been used for the manufacture of unique devices [6]. Over the past few years, there is a rapid development in the field of nanotechnology worldwide due to their wide applications in industry and biomedicine [1]. The toxicological concerns of these nanomaterials become subject of concern because of their distinct properties, such as small size [7], high number per given mass and large specific surface area [8], free radicals generation and their transportations through cell membrane into the mitochondria [6]. It is observed that the potential for some nanomaterials to be toxic for humans or the environment [9,10].

Over various types of nanomaterials and subcategories, the carbon nanotubes (CNTs) belong to the nanomaterials family [11]. CNTs comprising single-walled and multi-walled carbon nanotubes (SWCNTs and MWCNTs) composed of single or multiple graphene sheets rolled into cylinders and make up a complex family [12]. The exceptional physico-chemical (structural, electrical, mechanical, electromechanical and chemical) properties of CNTs make it prominent materials over other materials. These properties make its valuable for various applications such as nano electronics, sensors, electrochemical, storage of energy because of their unique morphology [13,14]. The toxicity of CNTs is attributed to their physico-chemical characteristics, such as length, diameter, shape, purity, surface area and surface chemistry [13–15]. Towards physico-chemical application of nanostructures aerographites were prepared via CVD process with using graphite on porous zinc oxide (ZnO) networks via flame transport synthesis process [16]. In another report, carbon based nanomaterials were used as a light...
An improved method of DNA preparation for PCR-based detection of *Brucella* in raw camel milk samples from Riyadh region and its comparison with immunological methods

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Abstract
Brucellosis a zoonotic disease is endemic in Saudi Arabia especially in the Najed region due to the customary consumption of raw milk. For a detailed investigation of brucellosis in camel milk, 80 samples were collected from Riyadh region. Twenty-four and sixteen percentage of the samples were positive for *Brucella* when tested with Rose Bengal test and ELISA, respectively. Genomic DNA from the milk samples was prepared using a modified method presented in this study. *Brucella* was detected in camel milk using three different primer sets targeting *Brucella* 16S rRNA gene, *omp*2, and *IS*711 genes. Among the three primer sets, an F4/R2 set of primers targeting *Brucella* 16S rRNA gene was most sensitive and yielded PCR products from nine milk samples. Finally, the 16S rRNA gene sequences of these isolates confirmed the contamination of *Brucella* spp. in nine milk samples. This study, therefore, suggests a high incidence of *Brucella* in camels of Riyadh.

Practical applications
This manuscript describes a modified method for the isolation of DNA from camel milk. The DNA was subsequently used successfully for the amplification of various genes for detecting *Brucella*. Furthermore, there was no report from Riyadh region on the detection of *Brucella* from camel milk. This report, therefore, points out toward an important health issue associated with consumption of raw camel milk. If published, this report will be helpful in educating the local population about the health risk associated with the consumption of raw milk.

1 | INTRODUCTION
Brucellosis a global zoonotic disease (Dean, Crump, Greter, Schelling, & Zinsstag, 2012; Pappas, Papadimitriou, Akritidis, Christou, & Tsianos, 2006) is a matter of special concern to Saudi Arabia where the incidence in some region is as high as 8–8.8% (Ahmed & Ahmed, 2009; Memish, 2001). Dean et al. (2012) have reviewed that the incidence of brucellosis in the kingdom is much higher than other countries in the region. According to some estimates, more than 8,000 cases per year are reported to public health authorities (Memish & Mah, 2001). The incidence is particularly higher in rural areas due to the nomadic tradition of consuming raw milk (Wernery, 2014). Surprisingly, very few systematic reports on brucellosis are available from the Kingdom, central Asia, and Sub-Saharan Africa (Al-Freihi et al., 1986; Dean et al., 2012; Kiel & Khan, 1987). Although reports on human brucellosis from different regions of Saudi Arabia are available almost no report on camel brucellosis, one of the primary sources of the disease, were found in the literature from the region (Ahmed & Ahmed, 2009; Al-Sekait, 2000; Gwida et al., 2012). Brucellosis is caused by *Brucella* genus belonging to Alphaproteobacteria that currently comprises of six species (Moreno, Cloeckaert, & Moriyón, 2002). The two most prevalent species causing brucellosis in the Saudi Arabia are *Brucella melitensis* and *B. abortus* (Al-Eissa, 1999; Madkour, Mohamed, Talukdar, & Kudwah, 1985). It has also been demonstrated that the camels are sensitive to these two species of *Brucella* (Cooper, 1991). Therefore, it is both urgent and necessary to monitor the disease in the region, especially by controlling the source of the pathogen. Developing improved methods for economic, easy, and accurate detection of *Brucella* in camel or...
In vitro interaction of cefotaxime with calf thymus DNA: Insights from spectroscopic, calorimetric and molecular modelling studies

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Abstract

Cefotaxime is third generation antibiotic with known therapeutic efficacy against bacterial infections including cerebral abscesses and bacterial meningitis. The β-lactam group of drugs are considered safest antibiotics. Many antibiotics directly interact with DNA and alter their expression profile. Thus, it is necessary to understand the binding mode and its relevance to drug activity and toxicity. There is considerably a remarkable focus on deciphering the binding mechanism of these therapeutic agents as DNA is one of the major target for wide range of drugs. Cefotaxime has been extensively studied for its pharmacological properties while its binding mode to DNA has not been explicatd so far. In this study, we have unveiled the binding mechanism of cefotaxime to DNA by using various biophysical, thermodynamic and in silico techniques. UV–vis spectroscopy confirmed the formation cefotaxime–DNA complex along with a brief idea about the extent of interaction. Fluorescence spectroscopy yielded the values of various binding constants and explained mode of fluorescence quenching to be static. CD spectroscopy, thermal denaturation, KI quenching and viscosity measurement explained that cefotaxime is groove binder. Measuring the effect of ions on cefotaxime-DNA complex ensured that it does not bind to DNA electrostatically. Dye displacement experiments finally confirmed that cefotaxime binds to the minor groove of DNA. ITC gave the thermodynamic profile of this binding in which negative value of Gibb’s free energy change revealed that the process is spontaneous. Molecular modelling finally strengthened our experimental results that cefotaxime was located in curved contour of minor groove of DNA. The findings support on safety of drug and may have a little interference on normal biological functions.

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1. Introduction

Deoxyribonucleic acid (DNA) is the genetic material for inheritance which codes for the structure as well as function in living organisms and thereby making an obvious attention for researchers [1]. Since DNA is pharmacological target for enormous class of drugs including antibiotics, many drugs have been reported to control the transcription or replication. Therefore, the intensive research on characterization of binding mechanism of small molecules to DNA may lead to the development of effective therapeutic agents in modulating the gene expression [2]. Better understanding of interaction of these chemotherapeutic agents with DNA could result in unveiling the underlying molecular mechanism of action of drugs and will also help in assessing its safety at molecular level [3]. Small molecules such as drugs may interact with DNA via non-covalent or covalent binding. Non-covalent interactions include intercalative binding, groove binding and electrostatic interactions [4]. Groove binding may either be minor groove binding or major groove binding that are predominantly stabilized by van der Waals interaction and hydrogen bonds formed between small molecules and nitrogenous bases of DNA [5]. In intercalative mode of binding, a molecule gets inserted into the base pairs of opposite strands of DNA. The electrostatic interactions are formed between the negatively charged phosphate group of DNA and a positively charged small molecule [6]. Scientific studies revealing such interactions will assist in understanding the mechanism of action of these drugs at molecular level.

Cefotaxime is third generation antibiotic, belonging to cephalosporin class, commonly recommended for clinical therapy of bacterial infections including cerebral abscesses and bacterial meningitis [7]. It has been also documented to be used in food processing and preservation [8]. It has potent activity mainly against gram negative bacteria belonging to family enterobacteriaceae that may cause brain abscess, meningitis gonorrhoea etc [9]. Cefotaxime treatment on spermatogonial cells resulted in sperm head abnormalities showing the toxic behaviour of this antibiotic.
Inhibitory effect of vitamin B₃ against glycation and reactive oxygen species production in HSA: An in vitro approach

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Hyperglycaemia is a key factor for the formation of advanced glycated endproducts (AGEs). Inhibition of glycation may play key role in minimizing the diabetes related complications. We have tried to explore the glucose and methyl glyoxal mediated glycation and antiglycation activity of niacin using human serum albumin as model protein. Protein was incubated with glucose for 28 days at physiological temperature to achieve glycation. Antiglycation activity was evaluated by assessing free lysine, carbonyl content, AGE specific fluorescence. Molecular docking and isothermal titration calorimetry was deployed to study the interaction of niacin with HSA and get a detailed insight of binding site and thermodynamics of interaction. Niacin reduced the glycation significantly which was evident from the estimation of free lysine and carbonyl content. Niacin binds with HSA in a spontaneous manner with the binding constant in the range of 10⁶ M⁻¹. Niacin also prevented the loss in secondary structure induced by glycation. Niacin was found to be located at Sudlow’s site I with binding energy of 5.3 kcal/mol. These results clearly highlight the antiglycation activity of niacin and its potential in preventing disease progression in diabetes.

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1. Introduction

Glycation is a process in which free reducing sugars react non-enzymatically with free amino group of proteins DNA and lipids. The early unstable product is characterized as Schiff’s base that later results in formation a stable Amadori product [1]. This reaction proceeds by oxidative modifications and rearrangements triggering the proteins to cross-link and resulting in formation of advanced glycation endproducts (AGEs) [2]. Hyperglycaemia is associated with type 2 diabetes favours glycation and accumulations of AGEs in vivo. AGEs alter different signalling pathways leading to generation of oxidative stress as well as other severe pathological conditions including neuropathy, nephropathy, retinopathy and micro-vascular complications [3,4]. Once glycation starts, formation of AGEs continues for whole life span that cannot be stopped even after normalization of blood glucose level [5,6]. At normal blood glucose level, modification of 6–10% of lysine chains of human serum albumin (HSA) occurs non-enzymatically which is amplified up to three-folds under hyperglycaemia in diabetes [7]. The major in vivo AGEs characterized till date are product of highly reactive carbonyl intermediates which include glyoxal, methyl glyoxal, 3-deoxyglucosone pentosidine N-ε-carboxymethyllysine (CML) etc. collectively called as dicarbonyls or oxoaldehydes [8,9]. AGEs are highly reactive heterogenous class of compounds which are difficult to be characterized by single technique. Some specific properties of AGEs are exploited for their characterization such as fluorescence, specific antibodies, mobility in polyacrylamide gel depending on their cross-linking [10–12].

Type 2 diabetes is a major global health problem and 90% of its cases are related to defects in glucose metabolism [13]. The mechanisms involved in diabetes are required to be scrutinized at molecular, genetics and physiological levels. Irrespective of the advancements in diabetes treatment, the rate of mortality and morbidity related to the disease is still quite high. One of the known mechanisms responsible for tissue damage in diabetes is non-enzymatic glycosylation [14]. The AGEs formed gets accumulated in vascular tissues where they bind to AGE specific receptors called RAGE. Hyperglycaemia accelerates binding of AGEs to RAGE altering enzyme activity, immunogenicity and modify the half-life
Eugenol inhibits quorum sensing and biofilm of toxigenic MRSA strains isolated from food handlers employed in Saudi Arabia

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ABSTRACT

Food handlers are important component in assessment and maintenance of food quality as they are carriers of food pathogens causing spoilage. Food spoilage is attributed to quorum sensing (QS) controlled development of biofilms. Therefore, there is an urgent need to develop novel QS and biofilm inhibitors to prevent spoilage of food products. In the present study, toxin producing biofilm forming methicillin-resistant Staphylococcus aureus (MRSA) were isolated from food handlers. Further, eugenol was screened for its QS and anti-biofilm properties. Analysis of nasal and hand swabs revealed the presence of seven toxigenic and biofilm MRSA strains. Eugenol demonstrated significant anti-QS activity in CVO26 and also reduced the QS-regulated production of elastase, protease, chitinase, pyocyanin and exopolysaccharide (EPS) in PAO1 considerably. Eugenol demonstrated 17%–86%, 24%–69%, 30%–91%, 9%–94% and 4%–89% reduction in biofilm biomass of S. aureus ATCC 25923 and MRSA strains FSA3, FSA11, FSA13 and FSA32, respectively. Sub-inhibitory concentrations of eugenol also decreased the metabolic activity in biofilm cells. Molecular docking analysis showed high binding affinity of eugenol that represents its biofilm inhibitory activity. This is the first report on the carriage of toxigenic drug-resistant biofilm forming S. aureus by food handlers and inhibition of their biofilms in the Kingdom of Saudi Arabia. The findings give a clear insight into the food safety hazards associated with the carriage of S. aureus and present eugenol as a broad-spectrum anti-QS and anti-biofilm agent.

Introduction

Foodborne illness is one of the major health concerns worldwide and according to the estimates of World Health Organization (WHO) around 30% population in the developed countries suffers from food-related health hazards annually, whereas in the third world countries mortality of two million is estimated per year [1]. Food handlers are a common and persistent cause of the spread of foodborne diseases [2] and Staphylococcus aureus is one of the important pathogens often transmitted via food contaminated by an infected food handler.

Staphylococci are ubiquitously found in nature and are frequently isolated from food and environmental sources. Staphylococcus aureus is a foodborne pathogen that can cause localized and invasive infections in humans due to consumption of contaminated food [3]. The infection causing ability of S. aureus is attributed primarily to the production of staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST) [4]. Although toxin production is the major cause of the foodborne illness caused by the S. aureus, other factors like biofilm formation and antibiotic resistance also contribute to its pathogenicity. Drug-resistant staphylococci are a major public health concern as the bacteria can circulate easily in the environment through the food chain [5]. In the past two decades, infection caused by methicillin-resistant Staphylococcus aureus (MRSA) has increased considerably across the globe. Multi-drug resistant strains of S. aureus have been frequently recovered from food stuff and food handlers [6]. Staphylococci can also form biofilms on various surfaces and impart resistance to the pathogen against antibiotics and sanitizers [7]. Therefore, biofilm formation is a marker of pathogenicity for drug-resistant S. aureus [8]. Biofilm formation has been extensively studied in clinical settings, but limited information is available regarding staphylococcal biofilm in the food industry.

Food industry is currently facing severe problem of food spoilage and biofilm formation by foodborne pathogens and even with the application of modern
Leaf Extracts of *Mangifera indica* L. Inhibit Quorum Sensing – Regulated Production of Virulence Factors and Biofilm in Test Bacteria

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Quorum sensing (QS) is a global gene regulatory mechanism in bacteria for various traits including virulence factors. Disabling QS system with anti-infective agent is considered as a potential strategy to prevent bacterial infection. *Mangifera indica* L. (mango) has been shown to possess various biological activities including anti-QS. This study investigates the efficacy of leaf extracts on QS-regulated virulence factors and biofilm formation in Gram negative pathogens. Mango leaf (ML) extract was tested for QS inhibition and QS-regulated virulence factors using various indicator strains. It was further correlated with the biofilm inhibition and confirmed by electron microscopy. Phytochemical analysis was carried out using ultra performance liquid chromatography (UPLC) and gas chromatography–mass spectrometry (GC-MS) analysis. *In vitro* evaluation of anti-QS activity of ML extracts against *Chromobacterium violaceum* revealed promising dose-dependent interference in violacein production, by methanol extract. QS inhibitory activity is also demonstrated by reduction in elastase (76%), total protease (56%), pyocyanin (89%), chitinase (55%), exopolysaccharide production (58%) and swarming motility (74%) in *Pseudomonas aeruginosa* PA01 at 800 µg/ml concentration. Biofilm formation by *P. aeruginosa* PA01 and *Aeromonas hydrophila* WAF38 was reduced considerably (36–82%) over control. The inhibition of biofilm was also observed by scanning electron microscopy. Moreover, ML extracts significantly reduced mortality of *Caenorhabditis elegans* pre-infected with PA01 at the tested concentration. Phytochemical analysis of active extracts revealed very high content of phenolics in methanol extract and a total of 14 compounds were detected by GC-MS and UPLC. These findings suggest that phytochemicals from the ML could provide bioactive anti-infective and needs further investigation to isolate and uncover their therapeutic efficacy.

**Keywords:** *Mangifera indica*, leaf extract, quorum sensing, biofilm inhibition, *C. elegans*, GC-MS, virulence factors
Diabecon, an Ayurvedic Formulation Exhibits Antiglycation Effect in Vitro

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Abstract- Hyperglycaemia is the hallmark of diabetes that increases the pathophysiological complications in diabetes. Under hyperglycaemia, proteins react with sugars both enzymatically and non-enzymatically to form advanced glycation end products (AGEs) via a series of complex reactions. The AGEs formed under in vivo conditions generate free radicals and induces oxidative stress to various tissues and organs and thereby amplifies the diabetic complexities. Inhibition of glycation is one of strategy to avoid such complications. Various antidiabetic drugs, both modern and herbal medicines may exhibit varying level of antiglycation effect as one of the antidiabetic mechanism. However, many herbal formulations have not yet systematically explored for their mode of action on this aspect. In this study, we have investigated a polyherbal Ayurvedic formulation for Diabetes (Diabecon) for its potency in inhibiting protein glycation. BSA samples were incubated for 28 days with glucose in absence and presence of varying concentration (200-1000 µg/ml) of Diabecon under physiological conditions. The results showed that Diabecon significantly inhibited non-enzymatic glycation and protected BSA from unfolding. Inhibition of AGEs and fructosamine under in vitro conditions was recorded. These results highlight the role of Diabecon in inhibition of glycation and in turn preventing disease progression. The findings also validate the antidiabetic activity of Diabecon and proposed that its antiglycation mode of action may significantly contribute to its overall efficacy.

Keywords: Diabecon, BSA, Glycation, AGEs, Fructosamine, Diabetes

Introduction

Diabetes mellitus is a metabolic disorder which is characterized by abnormal carbohydrate, fat and protein metabolism that ultimately affects insulin secretion as well as insulin action (Alam et al., 2016). Earlier, the disorder was regarded as disease of the rich and affluent while this has become a chronic disease irrespective of geographic location and socioeconomic status. It is one of the leading causes of death in developing and developed countries producing a substantial evidence of becoming an epidemic mainly in developing nations (Whiting et al., 2011). Hyperglycaemia is hallmark of diabetes whether type I or type II. Elevated blood sugar causes glycation of large number of proteins in which human serum albumin (serum protein) is mainly affected. Glycation is a reaction of reducing sugars like glucose and fructose with protein and nucleic acids that give rise to an unstable Amadori products or Schiff’s base (Qais et al., 2016). Proteins are more prone to glycation than nucleic acids. These unstable glycation intermediates further follow complex cascade reactions that include condensation, rearrangements, oxidative modifications causing the abnormal cross-linking of proteins and ultimately forms advanced glycation end products (AGEs) (Brownlee, 2001). Under in vivo conditions, methylglyoxal, glyoxal and dicarbonyls are major classes of AGEs formed. The accumulation of AGEs in body tissue leads to increase in pathophysiological complications associated with diabetes. This stimulates the production of reactive oxygen species (ROS) causing massive tissue damage. The herbal medication has recently attained a considerable popularity and acceptance
Interaction of capsaicin with calf thymus DNA: A multi-spectroscopic and molecular modelling study

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Studying the mode of interaction between small molecules and DNA has received much attention in recent years, as many drugs have been reported to directly interact with DNA thereby regulating the expression of many genes. Capsaicin is a capsaicinoids family phytocompound having many therapeutic applications including diabetic neuropathy, rheumatoid arthritis, prevention of DNA strand breaks and chromosomal aberrations. In this study, we have investigated the interaction of capsaicin with calf thymus DNA using a number of biophysical techniques to get an insight and better understanding of the interaction mechanism. Analysis of UV-vis absorbance spectra and fluorescence spectra indicates the formation of complex between capsaicin and Ct-DNA. Thermodynamic parameters ΔG, ΔH, and ΔS measurements were taken at different temperatures indicated that hydrogen bonding and van der Waal’s forces played major role in the binding process. Additional experiments such as iodide quenching, CD spectroscopy suggested that capsaicin possibly binds to the minor groove of the Ct-DNA. These observations were further confirmed by DNA melting studies, viscosity measurements. Molecular docking provided detailed computational interaction of capsaicin with Ct-DNA which proved that capsaicin binds to Ct-DNA at minor groove. Computational molecular docking also revealed the exact sites and groups to which capsaicin interacted.

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1. Introduction

A living organism has genetic material containing the coded information for its functioning that makes the DNA an obvious target of study for many researchers [1,2]. The drug-DNA interaction is an important area since it provides valuable information in the development of drugs and controlling gene expression [3–5]. DNA is also target molecule for many drugs including those under advanced clinical trials, especially anticancer drugs [6,7]. Small molecules like drugs interact with DNA via mainly three different binding modes: intercalation, groove binding and ionic interactions [8]. Many such molecules may directly interact with DNA and the factors controlling these interactions are still not very well understood. Studying these interactions has become simpler due to the availability of well-known three-dimensional structure of DNA, availability of genomic sequence and many automated computer programs. Studying these interactions also enables us to understand the mechanism of action of drugs at molecular level.

Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and dihydrocapsaicin constitute upto 90% of total capsaicinoids in which capsaicin accounts for approximately 71% [9]. Capsaicins have many therapeutic applications including those in diabetic neuropathy and rheumatoid arthritis [10]. This is one of the important dietary phytocompound having not only anti-carcinogenic effects [11], but has also been reported to prevent DNA strand breaks and chromosomal aberrations [12]. Studies have revealed that capsaicin inhibits the activity of ethylmorphine-¿ demethylase and many drug metabolizing enzymes of liver by interacting with cytochrome P-450 [13]. On the contrary, it is also evident that capsaicin has tumor promoting effects and people consuming large amount of chilli peppers are more prone to get gastric cancer [14,15].

Such compound needs to be investigated to get insights into their molecular mechanism of interaction with DNA. Therefore, in the present study, we have tried to explore the mode of interaction between capsaicin and Ct-DNA by using various biophysical and molecular modelling techniques. In silico molecular modelling complemented the in vitro interactions results and confirmed binding mode as minor groove binder.

Abbreviations: Ct-DNA, calf-thymus DNA; AO, acridine orange; EB, ethidium bromide; KI, potassium iodide; CD, circular dichroism.
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Impact of Metal Oxide Nanoparticles on Beneficial Soil Microorganisms and their Secondary Metabolites

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ABSTRACT - In this study, the effect of ZnO and TiO₂-NPs on beneficial soil microorganisms and their secondary metabolites production was investigated. The antibacterial potential of NPs were determined by growth kinetics of P. aeruginosa, P. fluorescens and B. amyloliquefaciens. Significantly decreased in the cell viability based on optical density measurements were observed upon treatment with increasing concentrations of NPs. While comparing the effect of the different concentrations of the NPs (200 µg/ml) on IAA production by different bacterial strains, ZnO nanoparticles showed greater inhibitory effect than TiO₂-NPs on IAA production by bacterial strains. The effect of Nanoparticles on phosphate solubilization was found inhibitory at 200 µg/ml. Treatment with ZnO showed concentration dependent enhancement in siderophore production by bacteriaby exposure to ZnO-NPs whereas TiO₂-NPs showed concentration dependent progressive decline for iron binding siderophore molecules. Reduction in antibiotic production by P. aeruginosa and P. fluorescens was noticed in the presence of ZnO and TiO₂ as compared to the control. The fluorescence of NADH released by P. aeruginosa was observed to be quenched in presence of ZnO and TiO₂-NPs as compared to control. The present study highlights that the impact of nanoparticles on bacterial strains and the release of plant growth promoting substances by PGPR strains was dose dependent, which gives an idea about the level of toxicity of these nanoparticles in the environment. Therefore, the discharge of nanoparticles in the environment should be carefully monitored so that the loss of both structure and functions of agronomically important microbes could be protected from the toxicity of MO-NPs.

Key-words- MO-NPs, IAA, Phosphate Solubilization, Siderophore, PCA, NADH, ZnO-NPs, TiO₂-NPs

INTRODUCTION
Nanotechnology manipulates the enhanced reactivity of materials at the atomic scale for the advancement of various applications for humankind. Various metal oxides nanoparticles, due to their optical, electrical and magnetic properties[1] have numerous applications including sensors, catalysis, biomedical diagnostics and environmental remediation[2-4]. Since engineered nanoparticles (ENPs) released to the environment go down the soil, the effects of ENPs on soil processes and the organisms that carry them out should be grasped.
Synthesis and antimicrobial evaluation of fatty chain substituted 2,5-dimethyl pyrrole and 1,3-benzoxazin-4-one derivatives

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KEYWORDS
Condensation; Cyclization; Acetonyl acetone; Anthranilic acid; Structure activity relationship

Abstract Fatty acids themselves have a number of biological properties and its easy intake by the human body will focus to the synthesis of many heterocyclic moiety substituted with fatty acid residue, to make more gradual intake of heterocycles in the human body. 2,5-Dimethyl pyrrole 2(a–e) and 1,3-benzoxazin-4-one 4(b–e) derivatives were synthesized, from cyclization of fatty acid hydrazide 1(a–e) with acetonyl acetone and from the reaction of fatty esters 3(b–e) with anthranilic acid in the presence of POCl3, respectively. All these compounds were characterized with the help of IR, 1H NMR, 13C NMR and mass spectra. The synthesized compounds were screened for antimicrobial evaluation against gram-positive (Staphylococcus aureus SA 22, Bacillus subtilis MTCC 121), gram-negative (Escherichia coli K12, Klebsiella pneumoniae) and fungal strains (Candida albicans IOA-109) and were found to be good antimicrobial agents.

1. Introduction
The pyrrole ring is a part of many biological compounds such as the enzyme catalase, the bile pigment bilirubin and the mould pigment prodigiosin; it is also a significant part of macrocyclic porphyrin ring system of chlorophyll and hemin [1,2]. Apart from these properties pyrrole and its derivative possess a number of biological activities such as antiallergic, antitumor [3], antibacterial, antifungal [4], antiinflammatory, analgesic [5], anticonvulsant [6], antimycobacterial [7] antitubercular, anticancer [8] and anti HIV [9]. Substituted dimethyl pyroles can be synthesized from the widely used Knorr pyrrole synthesis [10]. Other methods are also known for the synthesis of 2,5-dimethyl pyrrole derivatives [11,12]. Sometimes for the synthesis of substituted pyroles, photochemical reactions are also used, which involves the use of other pyrrole precursor including the migration of group from one nitrogen atom to the ring carbon atom [13]. Despite the biological use of substituted dimethyl pyroles they have been synthetically...
Toxicity evaluation of textile effluents and role of native soil bacterium in biodegradation of a textile dye

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Abstract
Water pollution caused by the discharge of hazardous textile effluents is a serious environmental problem worldwide. In order to assess the pollution level of the textile effluents, various physico-chemical parameters were analyzed in the textile wastewater and agricultural soil irrigated with the wastewater (contaminated soil) using atomic absorption spectrophotometer and gas chromatography-mass spectrometry (GC-MS) analysis that demonstrated the presence of several toxic heavy metals (Ni, Cu, Cr, Pb, Cd, and Zn) and a large number of organic compounds. Further, in order to get a comprehensive idea about the toxicity exerted by the textile effluent, mung bean seed germination test was performed that indicated the reduction in percent seed germination and radicle-plumule growth. The culturable microbial populations were also enumerated and found to be significantly lower in the wastewater and contaminated soil than the ground water irrigated soil, thus indicating the biotic homogenization of indigenous microflora. Therefore, the study was aimed to develop a cost effective and ecofriendly method of textile waste treatment using native soil bacterium, identified as Arthrobacter soli BS5 by 16S rDNA sequencing that showed remarkable ability to degrade a textile dye reactive black 5 with maximum degradation of 98% at 37 °C and pH in the range of 5–9 after 120 h of incubation.

Keywords Degradation · Effluents · Heavy metals · Pollution · Textile industry · Toxicity

Introduction
Industrialization, despite being an important factor for economic development, is a major cause of environmental deterioration. The large amount of industrial by-products released into the environment contain some hazardous compounds which are deleterious to all forms of life. Textile industry uses a wide range of chemicals, including dyes which is an important constituent, during different steps of textile manufacturing (Qin et al. 2007). Therefore, enormous amount of liquid waste generated by this industry contain primarily the recalcitrant dyes and other hazardous chemicals like heavy metals and sulfides (Lotito et al. 2014). Textile industry consumes large amount of potable water, i.e., for the production of 1 kg of textile about 200 L of water are used and as a result, it discharges a lot of wastewater in various water bodies generally without any prior treatment that results in serious water pollution (Panda et al. 2006). Textile wastewater release is directly or indirectly related with various human illnesses and environmental pollution. The presence of dye color in water bodies is not only an esthetic problem, but also prevents sunlight penetration through the water surface, thus disturbing the ecosystem (Joshi et al. 2010).

Textile wastewater has a highly diverse composition containing a large number of organic and inorganic compounds like acids, dying bases, hypochlorite of sodium, hydrogen peroxide, peracetic acid, dyestuff, optical bleachers, finishing chemicals, starch and salts of heavy metals etc. (Spagni et al. 2012). Heavy metals are non-biodegradable and accumulate in human organs through food chain causing severe health implications (hemorrhage, diarrhea, dermatitis, liver and kidney malfunctioning, neuro-muscular and central nervous system disorder etc.) (US EPA 1999; Kaur and Mehra 2012).

Although, various methods have been employed for the toxicity assessment of industrial wastewater using both
Selection and characterization of *Bacillus thuringiensis* strains from northwestern Himalayas toxic against *Helicoverpa armigera*

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**Abstract**

In this study, we present the selection and the characterization of *Bacillus thuringiensis* (Bt) strains with respect to their cry/cyt gene content and toxicity evaluation toward one of the most important polyphagous lepidopteran pest, *Helicoverpa armigera*. Fifty-six Bt isolates were obtained from 10 different regions of northwestern Himalayas, recording a total *B. thuringiensis* index of 0.62. Scanning electron microscopy revealed presence of bipyramidal, spherical, flat and irregular crystal shapes; SDS-PAGE analysis of spore-crystal mixtures showed the prominence of 130, 70, and 100 kDa protein bands in majority of the isolates; PCR analysis with primers for eight cry and cyt gene families and 13 cry gene subfamilies resulted in isolates showing different combinations of insecticidal genes. Strains containing cry1 were the most abundant (57.1%) followed by cry12 (46.42%), cry11 (37.5%), cry2 (28.57%), cry4 (21.42%), cry1 (19.64%), cry3 (8.9%), and cry7, 8 (7.14%). A total of 30.35% of the strains did not amplify with any of the primers used in this study. Median lethal concentration 50 (LC50) estimates of spore-crystal mixtures of Bt-JK12, 17, 22, 48, and 72 against second instar larvae of *H. armigera* was observed to be 184.62, 275.39, 256.29, 259.93 μg ml⁻¹, respectively. *B. thuringiensis* presents great diversity with respect to the presence of crystal protein encoding genes and insecticidal activity. Four putative toxic isolates identified in this study have potential application in insect pest control. *B. thuringiensis* isolate JK12 exhibited higher toxicity against *H. armigera* than that of *B. thuringiensis* HD1, hence can be commercially exploited to control insect pest for sustainable crop production. The results of this study confirm the significance of continuous exploration of new Bt stains from different ecological regions of the world.

**KEYWORDS**

*Bacillus thuringiensis*, Bioassay, Cry proteins, *Helicoverpa armigera*, LC50, PCR

1 | INTRODUCTION

The escalating public concern, stringent environmental regulations, and build-up of resistant insect populations to synthetic pesticides have led to an increased interest in alternative eco-friendly pest control strategies. One of the most successful substitutes to the manmade pesticides is the use of entomopathogenic bacterium, *Bacillus thuringiensis* (Bt). The entomopathogenic potential of Bt is primarily due to its
Characterization of lepidopteran-specific cry1 and cry2 gene harbouring native Bacillus thuringiensis isolates toxic against Helicoverpa armigera

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A R T I C L E   I N F O

Keywords:
Bacillus thuringiensis
Lepidopteran-specific cry genes
Helicoverpa armigera

A B S T R A C T

Bacillus thuringiensis (Bt) based biopesticides are feasible alternatives to chemical pesticides. Here, we present the distribution of lepidopteran-specific cry1 and cry2 genes in native B. thuringiensis. Forty four out of 86 colonies were found to harbour crystals by phase contrast microscopy exhibiting a Bt index of 0.51. PCR analysis resulted in the amplification of cry1 in 24 and cry2 in 14 isolates. Twelve of the isolates showed presence of both cry1 and cry2, while 18 isolates did not show presence of either of the genes. Toxicity screening using spore-crystal mixtures against 2nd instar larvae of Helicoverpa armigera revealed that the isolates (50%) were either mildly toxic or not toxic (36.36%), and only 13.63% were toxic. The results are interesting, particularly so because the same isolates were previously reported to contain lepidopteran specific vip3A genes also, hence can complement the toxicity of the isolates harbouring vip3A genes.

1. Introduction

As the world’s population is increasing geometrically, achieving global food security-making safe and nutritious food accessible to everyone, and achieving so sustainably is a challenging task. Feeding estimated 9.2 billion people in 2050 would require raising overall food production by about 70% [1]. A major bottleneck in achieving this challenge is the competition from the insect pests. Insect pests are responsible for destroying one fifth of the world’s total crop production annually, leading to heavy economic losses. The major damaging insect pests of crops belong to the order Lepidoptera [2] and Helicoverpa armigera is one of the most significant lepidopteran pests with potential to attack more than 180 species of plants [3]. It is widely distributed in Asia, Europe, Africa and Australasia causing damages worth 2 billion US dollars annually, excluding the socio-economic and environmental costs associated with the use of chemical insecticides and the introduction of GM crops [4–6]. H. armigera has over the years developed resistance to various chemical insecticides [7,8] and of late, its resistance to genetically modified crops expressing insecticidal protein from B. thuringiensis has also been reported [9,10].

The most common method to control insect pest populations is the use of chemical insecticides. Two of their properties, long residual action and toxicity to a wide spectrum of organisms made chemical insecticides very useful against insect pests. However, extended use of certain chemical insecticides have caused many environmental problems like persistence, toxicity to non-target organisms including humans and development of insect resistance [11,12]; reviewed in [13]. One of the most promising alternatives to the man-made chemical pesticides is the use of natural insect pathogen, Bacillus thuringiensis (Bt). The entomopathogenic potential of Bt is primarily due to its ability to produce insecticidal crystalline proteins (Cry and Cyt) [14] and in certain cases due to the production of vegetative insecticidal proteins (Vips) [15]. The crystalline and vegetative insecticidal proteins are respectively produced during the sporulation and vegetative stages of Btgrowth. Upto November 2016, seventy four classes of Cry proteins (Cry1-Cry74), three classes of Cyt proteins (Cyt1-Cyt3) and four classes of Vip proteins (Vip1-Vip4) have been designated based on their amino acid sequence homology [16]. These toxins are highly specific in action, harmless to humans and other vertebrates and are biodegradable. Presently, there are more than 50,000 known strains of B. thuringiensis isolated from diverse environments around the world [17,18]. These strains exhibit varying degree of toxicity against different pests. Despite the availability of such large collection of B. thuringiensis strains and their insecticidal genes, three events have rendered the search for novel insecticidal strains GENES more urgent. First, a significant number of pests are not controlled with the available Cry proteins. Second, at times the level of expressed toxins is not high enough to kill the host and third, after many years of successful use in the field, the first cases of resistance to B. thuringiensis have appeared [19].
Mutagenicity of Wastewater Extracts from Pulp and Paper Industry

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Abstract

Wastewater samples were collected from pulp and paper mill located in Ka-shipur (India) and were extracted using dichloromethane (DCM), chloroform and hexane solvents (all were HPLC-grade). The extracts were assayed for their mutagenic potential using Ames Salmonella mutagenicity assay. TA98 strain was found to be the most responsive, in terms of induction factor (Mi) and slope (m) of the initial linear dose-response curve as determined by linear regression analysis up to the increasing doses indicating the presence of frame shift mutagens in the test samples. Mutagenicity of different extracts is arranged as follows: dichloromethane extracted water samples > hexane extracted water samples > chloroform extracted water samples. Hexane extract exhibited maximum mutagenic index of 13.0 and induction factor (Mi) 2.48 with TA98. The order of responsiveness based on the mutagenic index and induction factor for the test samples was in the following order: TA98 > TA97a > TA100 > TA102 > TA104. Our findings suggest that TA97a, TA98, TA100, TA102, TA104 were sensitive towards the wastewater extracts and showed considerable mutagenicity.

Keywords

Mutagenicity, Pulp and Paper Industry, Wastewater Extracts

1. Introduction

Pulp and paper industries are water intensive industry and release effluent that contain a variety of naturally occurring polymers such as poly aromatic hydrocarbon, tannins, fatty acids, resin acids, lignin and its derivatives which are known for their resistance to degradation [1]. The chemicals formed during the process of pulping, bleaching and paper making have deleterious effect on aquatic life and they ultimately bioaccumulate and biomagnify in the food chain. Several studies have reported impaired liver and reproductive dysfunction in
Active Constituents of Essential Oil Inhibit Growth and β-lactamase Production in Drug Resistant Pathogenic Bacteria
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Abstract- Development of multidrug resistant in pathogenic bacteria and slow discovery of new antibiotics has triggered interest in search of new anti-infective compounds from medicinal plants. β-lactamases are the groups of hydrolytic enzymes responsible for resistance to widely used β-lactam antibiotics. In this study we report antibacterial activity of four active compounds of aromatic plants namely Eugenol, Citral, Geraniol and Thymol, exhibited to MDR strains of Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus. These phytocompounds showed varying level of antibacterial activity with zone of growth inhibition varying from 10.28 ± 0.34 to 22.83 ± 0.76 mm. Klebsiella sp. were relatively less sensitive than E. coli and S. aureus. MIC data also revealed the similar pattern of sensitivity against phytocompounds. Overall, eugenol was found more effective compared to other compounds. Further, effect of these compounds on β-lactamase production by test bacteria was assessed and found that at sub-MIC concentration, eugenol (0.4%v/v) could inhibit the production of β-lactamase in more than one strain tested. The findings revealed that in addition to antibacterial activity, essential oil’s phytocompounds at non-inhibitory concentrations (sub-MICs) demonstrate anti-resistance activity by inhibiting the synthesis of β-lactamase. Further investigation is needed to explore the efficacy of phytocompounds against different types of β-lactamases and other resistance mechanism in bacteria.

Keywords: β-lactamases, Essential oil, Eugenol, Klebsiella pneumoniae, Phytocompounds,

Introduction
The continuous increase in new and modified modes of antimicrobial resistance in pathogenic microbes leads to serious public health problems and imposes a global risk (Molton et al., 2013). Unprecedented selection pressure and slow progress in the development of new antimicrobials gradually results in increase prevalence of multidrug resistance pathogenic bacteria including microbial resistance to β-lactam antibiotics (Ahmad and Aqil, 2007). Bacteria develops resistance by mutation and resistance gene acquisition. Different mechanism of resistance are present in bacterial cell. Inactivation of antibiotic by producing hydrolytic enzymes e.g. β-lactamases, is the most common mechanism against β-lactam antibiotics (Dale-Skinner and Bonev, 2008). β-lactamase production is also under control of transmissible plasmids and hence aggravation of antimicrobial resistance among the bacterial populations has been a major concern (Bush, 2002, Orhan et al., 2011; Maheshwari et al., 2016a, Maheshwari et al., 2016b).

Targeting β-lactamase with enzyme inhibitors along with combinational therapy of β-lactam drugs constitute the most common and successful chemotherapeutic approach (Rolinson, 1991). The most common clinically relevant β-lactamase inhibitors like clavulanic acid, tazobactum and sulbactum are successfully used against β-lactamase mediated resistance in combination with β-lactam antibiotics (Bebrone et al., 2010). However, these first generation inhibitors are
Growth stimulation and management of diseases of ornamental plants using phosphate solubilizing microorganisms: current perspective

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Abstract Ornamental plants play an important role in human society since flowers are considered a vital component due to their beauty, texture, color, shape and fragrance. To produce high quality ornamentals, growers in general have intensified the use of agrochemicals without considering their deleterious impact on floral attributes. Also, the agrochemicals (including fertilizers and pesticides) used in floriculture are expensive and their excessive application results in emergence of pathogens resistant to such chemicals. It has, therefore, become imperative to develop renewable, inexpensive and eco-friendly fertilizers without producing any disturbing impact on quality of ornamentals. In this regard, phosphate solubilizing microorganisms (PSM) among plant growth promoting rhizobacteria have been identified as an efficient alternative to agrochemicals in floriculture. Even though, there are adequate reports on the effect of PSM on growth and development of numerous plants, information on the impact of PSM on production and quality of ornamental plants is, however, critically scarce. Considering these gaps and success of PSM application in floriculture achieved so far, efforts have been directed to highlight the impact of PSM on the production of ornamentals grown distinctively in different production systems. Also, the role of PSM in the management of ornamental diseases is discussed and considered. The review will conclude by identifying several PSM for future researches aiming to improve the health and quality of ornamentals grown in different production systems. Use of PSM is also likely to reduce the use of chemicals in floriculture.

Keywords Ornamental plants · Floriculture · Phosphate solubilizing microorganisms · Disease management

Introduction

Ornamental plants in general are plants that are grown for decorative purposes, cut flowers and for aroma. Ornamental plants are also grown for showy foliage which may be deciduous, turning bright orange, red, and yellow or evergreen. Other ornamental plants are cultivated for their blooms. Due to these features, ornamental plants are considered extremely important in human society because flowers gratify humans by their beauty, texture, color, shape and fragrance. Globally, ornamental plants also serve as a main source of export materials and add value to the economy of the country. For example, Chrysanthemums, often called as mums or chrysanth, is one of the leading commercial flower due to its variable colors. Ornamental plants also possess medicinal value and are used to combat certain human diseases. As an example, Chrysanthemum tea, made from white and yellow Chrysanthemum flowers is used against influenza. Also, the extract of Chrysanthemum plants (stem and flower) have a wide variety of potential medicinal properties including anti HIV-1, antibacterial and antymycotic (Karishma et al. 2013a). Considering such an exquisite and varied importance of ornamental plants, there is need to enhance their—(1) growth, (2) flower production and (3) quality attributes such as number, longevity and size of flowers, which in effect is likely to give a significant monetary benefit to

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Effects of Plant Growth Promoting Rhizobacteria on the Performance of Greengram under Field Conditions

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Abstract

Despite reports on effects of single Plant Growth Promoting Rhizobacteria (PGPR) inoculation on legumes, response of greengram to combined inoculation with phosphate solubilizing asymbiotic Azotobacter and ACC deaminase positive symbiotic Bradyrhizobium sp. (vigna) under field conditions remains unexplored. The present study aims at identifying ACC deaminase producing and phosphate solubilizing bacterial strains and to assess their impact on greengram plants in order to find efficient and friendly co-cultures for developing effective bioinoculants for increasing sustainable production of legumes. Additionally, plant growth promoting activities of Azotobacter and Bradyrhizobium sp. (vigna) were determined using standard methods. The isolated bacterial cultures were characterized morphologically, culturally and biochemically and were identified as Bradyrhizobium sp. (vigna) and Azotobacter chroococcum. Dry matter accumulation in whole plants, symbiotic attributes, nutrient uptake and grain yields were significantly enhanced following co-inoculation of A. chroococcum and Bradyrhizobium sp. (vigna). The inoculation of Azotobacter with Bradyrhizobium increased seed yield by two fold and produced the highest grain protein. A- 75% and 52% increase in P concentration in root and shoot, respectively was observed for A. chroococcum, while P uptake was highest (0.52 mg/g) in shoots following combined inoculation of A. chroococcum with Bradyrhizobium at harvest. The highest N concentration in roots and shoot at harvest were observed with co-culture of A. chroococcum and Bradyrhizobium sp. (vigna). Gram negative Azotobacter and Bradyrhizobium solubilized insoluble phosphate, synthesized indole acetic acid, ammonia, cyanogenic compounds and exopolysaccharides while only Bradyrhizobium showed ACC deaminase activity. The results suggest that two unrelated bacteria belonging to symbiotic and asymbiotic group and capable of facilitating greengram production under field conditions and expressing multiple plant growth promoting activity can be used to produce composite bioinoculants for enhancing greengram production while saving the use of fertilizers.

Keywords: Azotobacter, ACC Deaminase, Bradyrhizobium, Greengram, Nutrient Uptake, Nodule, Seed Yield.

1. Introduction

In high input agricultural practices, chemical fertilizers are frequently used to optimize crop production. These expensive chemicals, however, when used injudiciously, have resulted in loss of soil fertility and consequently, the crop production (Lemanski and Scheu, 2014). Due to these reasons, focus in recent times has been shifted towards the use of inexpensive natural resources such as Plant Growth Promoting Rhizobacteria (PGPR): soil bacteria that colonize the roots of plants following inoculation onto seeds and that enhance plant growth (Kloepper and Schroth, 1978). The PGPR involving free living (asymbiotic) growth promoting rhizobacteria (Lutgenberg and Kamilova, 2009; Bhattacharya and Jha, 2012), symbiotic rhizobia (Ahmad et al., 2013; Peix et al., 2015) and phosphate solubilizers (Zaidi et al., 2009; Nosrati et al., 2014) have been used for enhancing the production of different crops (Mohite, 2013; Viruel et al., 2014) including legumes (Noreen et al., 2012; Singh et al., 2013). Among non-nodule forming diazotrophs, Azotobacter, a free living nitrogen fixer, discovered and described in 1901 by the Dutch Microbiologist and botanist Martinus Beijerinck, play an important role in crop improvement by supplying mainly nitrogen (N) to plants. However, apart from providing N to plants, Azotobacter promotes plant growth directly by secreting considerable amounts of biologically active substances like B vitamins, nicotinic acid, penothenic acid, biotin, gibberellic acid, Indole-3 Acetic Acid (IAA) and cytokinin (Ahmad et al., 2005; Lenin and Jayanthi, 2012; Oskar et al., 2014) and ammonia (Narula and Gupta, 1986) or indirectly by protecting the plant from diseases (Saini, 2012). Also, the secretion of l-aminocyclopropane-1-carboxylate (ACC) deaminase by PGPR including nitrogen fixers have been found to reduce the level of plant stress hormone ethylene and consequently to enhance plant growth (Akhgar et al., 2014; Hassan et al., 2014; Magnucka and Pietr, 2015). The
Heavy Metals: Biological Importance and Detoxification Strategies

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Abstract

Global modernization is responsible for industrialization, urbanization and several other anthropogenic activities, which involves the huge application of heavy metals. Heavy metals containing products during process and after disposal, release various heavy metals and ions in surrounding atmosphere which severely affect the soil and water quality. Heavy metal contaminated soils in general are nutrient deficient and are likely to become barren in future. If heavy metal polluted soil is used for crop cultivation then the heavy metals deposited in soil enter into food chain and at higher concentration create severe human health problems. On the contrary, at permissible limit, metals are important for enzymatic activity and genetic material integrity in biological system. To understand the importance and risk associated with heavy metals, a genuine attempt is made to present different aspects of metal contamination in soils. Furthermore, nutritive value of heavy metals and toxicity to bacteria and plants is discussed. Finally, different strategies adopted by biological systems to detoxify heavy metals are critically highlighted. This review is likely to help to better understand the over does risk of heavy metals and its biological detoxification strategies.

Keywords: Heavy metals; Toxicity; Detoxification; Pollution; Biological importance

Introduction

Due to consistently increasing human populations, the current agricultural systems are under tremendous pressure basically for two reasons: (i) cultivable land is declining very rapidly and (ii) the human food demand is on the rise. Therefore, well-directed and concerted efforts are needed in order to use the full potential of agro-ecosystems efficiently and to overcome these problems. However, the plant nutrients like nitrogen (N), phosphorus (P), potassium (K), and some other minor nutrients play important roles in crop improvement in conventional agriculture practices. In contrast, the deficiency in even micronutrients, which are typically present at <100 mg kg\(^{-1}\) dry weight, may significantly limit the crop yields in many production systems [1]. Some of micronutrients such as Cu, Fe, Mn, and Zn, are essentially required for various physiological functions of plants and animals. Although the majority of plants require these elements in minimal quantities, agricultural soils are often deficient in one or more of these micronutrients. In general, the concentration of such nutritional contents in plant tissues falls below the optimum levels. There are also minor elements, known as trace elements, or other metalloids which play important roles in functioning of living organisms including those of microflora. In addition, these elements could also participate in other activities: (i) forming the structure of proteins and pigment (ii) redox processes (iii) regulation of the osmotic pressure (iv) maintaining the ionic balance and (v) acting as enzyme component of the cells [2,3]. Among these elements, Al, Co, Se, and Si play a role in promoting plant growth and may be essential for particular taxa [4]. Likewise, Zn plays a significant role in cellular division and amplification, protein synthesis, and contributes in carbohydrate, lipid, and nucleic acid metabolism [5]. On the other hand, structure and composition of microbiota [6] and plant growth [7-10] are reported to be significantly affected when the concentrations of such trace elements exceed the normal level. Moreover, the concentration of these trace elements also varies from soil to soil and/or region to region. For instance, multiple surveys conducted to determine the status of nutrient in agricultural soils in China and India revealed that Zn is commonly the most deficient micronutrient in soil. While the nutritional deficiency levels in Chinese soils were (%): Zn 51, Mo 47, Mn 21, Cu 7, and Fe 5 [11], the deficiency levels in Indian soils were: 49 Zn, 33 B, 12 Fe, 11 Mo, 5 Mn and 3 Cu [12]. Thereby, identifying the elements of soil nutrient pools and their consequential effect on both microbes and plants are necessary for enhancing crop production and plant nutritional value.

Source of heavy metal in soils

Heavy metals are defined as metals and metalloids having densities greater than > 5 g cm\(^{-3}\). Heavy metals may be found in soils naturally [13-15] or can be added to soils through anthropogenic activities (Figure 1). Natural sources of heavy metals (HM) such as volcanoes emissions transport of continental dusts, and weathering of metal-enriched rocks due to long exposure to air, greatly adds higher amounts of HM to soils [16]. In addition, HM can also contaminate the soil through other human activities, such as: (i) exploitation of mines and smelters (ii) application of metal-based pesticides and metal-enriched sewage sludge in agriculture [17,18] (iii) combustion of fossil fuel, metallurgical industries, and electronics, and (iv) military training and weapons, etc. [19]. The anthropogenic activities have been categorized into five groups: (i) metalliferous mining and smelting (e.g., As, Cd, Pb and Hg), (ii) industry (e.g., As, Cd, Cr, Co, Cu, Hg, Ni and Zn), (iii) atmospheric deposition (As, Cd, Cr, Cu, Pb, Hg and U), (iv) agriculture (e.g., As, Cd, Cu, Pb, Se, U and Zn), and (v) waste disposal (e.g., As, Cd, Cr, Cu, Pb, Hg and Zn). Agricultural practices such as excessive application of phosphatic fertilizers for optimum crop production [20], extensive and injudicious usage of toxic pesticides, and use of sewage sludge can result in soil pollution [21].

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CHARACTERIZATION OF PAENIBACILLUS DURUS (PNF16) A NEW ISOLATE AND ITS SYNERGISTIC INTERACTION WITH OTHER ISOLATED RHIZOBACTERIA IN PROMOTING GROWTH AND YIELD OF CHICKPEA

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ABSTRACT
Application of PGPR in crop production and protection is well known and can also contribute in reducing use of agrochemicals. However, the performance of PGPR is influenced by various biotic and abiotic factors. Isolating new efficient PGPR strain well adapted to local soil agroclimatic conditions is expected to perform with more consistency. In this study five selected rhizobacteria isolated previously were first tested in vitro for plant growth promoting (PGP) characteristics. One of the isolate PNF16 was identified as Paenibacillus durus by 16S rRNA gene sequence analysis. PNF16 alone and in combination with other rhizobacteria (Azotobacter (AZT3), Achromobacter (PNF1), Bacillus (Bc), Pseudomonas (Ps)) was tested for plant growth promoting effect under pot conditions in two consecutive years. PNF16 was found to produce 21.7 µg ml⁻¹ of indole acetic acid like substances, hydroxymate type of siderophores (Salicylate type 11 µg ml⁻¹ and benzoate type 6.5 µg ml⁻¹) and solubilized phosphate (405.33 µg ml⁻¹). PNF16 - Mesorhizobium combination was found significantly better compared to other combinations for growth parameters, nodulation and yield of chickpea over control. Similar study was also performed for other tested strain. Significant increase in plant growth (32%), nodulation (43%) compared to untreated control was recorded. Co-inoculation also showed synergy and increased the number of pods per plant, 1000-grain weight, dry matter yield, grain yield and protein content by 23%, 22%, 21%, 18% and 4.4% respectively, compared to control. The results indicated the potential usefulness of PNF16 alone and in combinations in enhancement of nodulation and stimulation of plant growth in chickpea and adapted to soil condition of the region.

Keywords: Paenibacillus; Mesorhizobium; PGPR, plant-microbe interaction, nodulation, chickpea

INTRODUCTION
Interactions between plants and micro-organisms in the rhizosphere can clearly affect crop yields. Rhizobacteria that benefit plant growth and development are called ‘PGPR’. The term ‘PGPR’ was introduced in 1978 by Kloeper and colleagues. Since then a large number of bacteria have been identified and reported as PGPR (Acetobacter, Achromobacter, Arthrobacter, Azorarcus, Azospirillum, Azotobacter, Bacillus, Burkholderia, Clostridium, Enterobacter, Flavobacterium, Frankia, Hydrogenophaga, Kluyvera, Microcoleus, Phyllobacterium, Pseudomonas, Rhizobium, Serratia, Staphylococcus, Streptomyces, and Vibrio) (Bashan et al., 2005). However commonly used PGPR in field application is limited to only few microorganisms. The plant growth promoting rhizobacteria may enhance plant growth either directly or indirectly. Direct mechanisms include (i) the ability to produce the plant growth regulators (indoleacetic acid, gibberellins, cytokinins and ethylene) (Glick, 2012), (ii) Asymbiotic N₂ fixation (Ahmad et al., 2008), (iii) Solubilization of mineral nutrient like phosphates (Taurian et al., 2010), Indirect mechanisms involve (i) antagonism against phytopathogens (Gururani et al., 2013), (ii) Production of siderophores (Haas and Défago, 2005), (iii) Production of extra cellular cell wall degrading enzymes for phytopathogens β,1-3 glucanase (Ribeiro and Cardoso, 2012), Chitinase (Ribeiro and Cardoso, 2012), (iv) Antibiotic production (Mazurier et al., 2009) and (v) cyanide production (Ribeiro and Cardoso, 2012). By modifying the microbial balance in the rhizosphere, PGPR can stimulate plant growth indirectly by inhibiting other deleterious microbes or root pathogens (Berendsen et al., 2012). On the other hand, diazotrophs are able to decrease or prevent the deleterious effects of plant pathogens mostly through the synthesis of antibiotic and fungicidal compounds (Mavingui and Healin 1994; Dobbeltaere et al., 2003), competition for nutrients (siderophore production) or by the induction of induced systemic resistance (ISR) against pathogens (Timmusk and Wagner 1999; Dobbeltaere et al., 2003; Gururani et al., 2013). A major problem associated with PGPRs is their inability to manifest PGP traits under natural field conditions consistently. This is mainly due to competition with native well adapted strains and specific nutrient limitation (Vasssey, 2003). We hypothesized that selecting a PGPR strains exhibiting multiple traits are expected to most ideal as the probability of expression of one or more PGP traits is higher. It is also expected that indigenous soil bacteria adapted to local soil and agro-climatic conditions exhibiting multiple PGP traits may be more effective under field conditions. We have screened rhizospheric soil in vicinity of Aligarh in northern India (Ahmad et al., 2006). We found a new isolate of Paenibacillus sp. (PNF16) which showed multiple PGP traits and characterized using 16S rRNA gene sequence analysis. The efficacy of PNF16 for plant growth promotion was assayed under pot experiment conditions alone and in combination with other bioinoculant such as Bacillus (Bc), Azotobacter (AZT3), Achromobacter (PNF1) and Pseudomonas (Ps) which were previously isolated in our laboratory.

MATERIALS AND METHODS
Isolation and characterization of bacterial isolates
Bacterial isolates PNF16 and other rhizobacteria used in this study were isolated and biochemically characterized using standard methods as described previously (Ahmad et al., 2006; 2008).

Genetic identification of PNF16 by 16S rRNA partial gene sequencing
Single isolated colony PNF16 was inoculated in 5 ml Luria-Bertani (LB) broth and grown at 30 °C for 24 h. Cells were harvested and processed immediately for DNA isolation by standard procedure. The concentration and purity of the DNA preparation were determined by measuring optical density (OD) at 260 nm and ratio at 260/280 nm with a UV-Vis Spectrophotometer. The PCR amplification of almost full-length 16S rRNA gene was carried out with eubacterial specific
Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates

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Abstract

ZnO nanoparticles (ZnONPs) were synthesised through a simple and efficient biogenic synthesis approach, exploiting the reducing and capping potential of Aloe barbadensis Miller (A. vera) leaf extract (ALE). ALE-capped ZnO nanoparticles (ALE-ZnONPs) were characterized using UV–Vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and transmission electron microscopy (TEM) analyses. XRD analysis provided the average size of ZnONPs as 15 nm. FTIR spectral analysis suggested the role of phenolic compounds, terpenoids and proteins present in ALE, in nucleation and stability of ZnONPs. Flow cytometry and atomic absorption spectrophotometry (AAS) data analyses revealed the surface binding and internalization of ZnONPs in Gram +ve (Staphylococcus aureus) and Gram –ve (Escherichia coli) cells, respectively. Significant antibacterial activity of ALE-ZnONPs was observed against extended spectrum beta lactamases (ESBL) positive E. coli, Pseudomonas aeruginosa, and methicillin resistant S. aureus (MRSA) clinical isolates exhibiting the MIC and MBC values of 2200, 2400 μg/ml and 2300, 2700 μg/ml, respectively. Substantial inhibitory effects of ALE-ZnONPs on bacterial growth kinetics,
Application of nanoparticles in oral hygiene

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ABSTRACT

Many of more than 700 bacterial species inhabiting the oral cavity are opportunistic pathogens causing systemic infections in addition to dental and periodontal diseases. This renders oral hygiene a much serious issue, which is further exacerbated with the emergence of multiple antibiotic resistance in oral bacteria. The role of nanoparticles based materials especially metal and metal oxide nanoparticles as an effective and alternative-supplementary antimicrobial agent is now well established. These nanoparticles could be a healthier, innocuous and effective alternative for controlling both the dental biofilms and oral planktonic bacterial population with lesser side effects or antibiotic resistance. Antimicrobial activity of these nanoparticles against a number of oral pathogens has already been demonstrated. When added to artificial dental materials and implants these nanoparticles improve the desirable physico-chemical properties of the materials in addition to improving their antimicrobial activity. Besides a few studies, biochemical processes underlying the antimicrobial activity of the nanoparticles against both planktonic cells and oral biofilms is not understood. Through our literature survey it is envisaged that ZnO nanoparticles and TiO₂ nanoparticles are the most suitable nanoantibiotic for the development of dental pastes, mouthwashes, and other oral hygiene materials. However in vivo studies on nanotoxicity of these nanoparticles are missing and need a careful and balanced evaluation before successful clinical translations.

Keywords: Nanostructures, Oral Hygiene, Bacteria, Toxicology, Pharmacology.

1. INTRODUCTION

Human oral microbiome is one of the most complex microbiomes, different studies estimate the presence of 25,000 different phylotypes or 700 different species of bacteria in the oral cavity (Jenkinson et al. 2011; Keijser et al. 2008; Liu et al. 2012; Paster et al. 2006).

This remarkable diversity of the microbial community can be attributed to the diverse micro-niches within the oral cavity, distinguished by their physico-chemical properties (Segata et al., 2012). The teeth, tongue, mucosa, palate, and gingiva harbor distinctive microbiota as found by both culture dependent and culture independent approaches (Aas et al., 2005; Jenkinson et al., 2011; Keijser et al. 2008; Liu et al. 2012; Paster et al., 2006). It is often difficult to culture all the microorganisms present in a niche due to the diverse and often unknown nutritional and physico-chemical requirements of the diverse bacteria present in a given niche.

Molecular-based, culture-independent techniques, such as pyrosequencing, and 16S rRNA profiling, have provided important new insights into the diversity of the microbiome within the oral cavity (Crielaard et al., 2011). Especially pyrosequencing allows an extensive and high-throughput characterization of microbial communities. In an interesting study using pyrosequencing it was observed that microbial community present even on two different sites of the same tooth varied significantly. For instance, the genera Streptococcus constituted 29% to 70% on the vestibular surfaces of teeth but on the sulcus side constituted only 5% to 21% in almost all the samples studied. While quadrants 1 and 2, displayed a higher percentage of Aggregatibacter and Capnocytophaga. This, diversity of microbial populations can be attributed to the heterogeneity of physicochemical properties of the micro-niches within the oral environments. Even the same tooth or teeth in close proximity were found to have different pH, oxygen levels, temperature, and redox potential (Kleinberg and Jenkins, 1964). These variations in physico chemical characteristics of micro-niches influence the colonization of micro-organisms (Fejerskov et al., 1994). Despite this diversity, a number of investigators have tried to identify bacteria involved in the oral diseases and to distinguish them from normal oral bacteria (Aas et al., 2005).

However, the biochemical conditions favoring the proliferation of pathogenic bacteria within the oral cavity leads to periodontitis, an inflammatory disease, which can also constitute a risk factor for other systemic diseases (Zbinden et al., 2012) such as endocarditis and colorectal cancer (Han et al., 2013). Therefore, it is both urgent and important to clarify the role of microbial communities in systemic diseases and human health (Belda-Ferre et al., 2011; Turnbaugh et al., 2007; Ximénez-Fyvie et al., 2000). The problem is further complicated by the development of resistance to traditional antibiotic in oral bacteria, which is not only limited to the human subjects receiving antibiotic therapies (Leistevuo et al., 2000; Sweeney et al., 2004).

The search for new, effective and economic alternative antimicrobial agents is therefore crucial to combat microbial infections. Metal oxide nanoparticles exhibit remarkable antimicrobial activity and are also referred to as nanoantibiotics (Huh and Kwon, 2011). Hence, the role of these nanoantibiotics to abate and/or control the growth of bacteria in oral cavity (Allaker, 2010) is proposed here. In this review, the impact of oral cavity
Synthesis, characterization of α-amino acid Schiff base derived Ru/Pt complexes: Induces cytotoxicity in HepG2 cell via protein binding and ROS generation

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A B S T R A C T

We have synthesized two new complexes of platinum (1) and ruthenium (2) with α-amino acid, L-alanine, and 2,3-dihydroxybenzaldehyde derived Schiff base (L). The ligand and both complexes were characterized by using elemental analysis and several other spectroscopic techniques viz; IR, 1H, 13C NMR, EPR, and ESI-MS. Furthermore, the protein-binding ability of synthesized complexes was monitored by UV–visible, fluorescence and circular dichroism techniques with a model protein, human serum albumin (HSA). Both the PtL2 and RuL2 complexes displayed significant binding towards HSA. Also, in vitro cytotoxicity assay for both complexes was carried out on human hepatocellular carcinoma cancer (HepG2) cell line. The results showed concentration-dependent inhibition of cell viability. Moreover, the generation of reactive oxygen species was also evaluated, and results exhibited substantial role in cytotoxicity.

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1. Introduction

From the ancient times, medicinal applications of metal salts can be sketched [1–3] with gold and silver like precious metals. However, the discovery of cisplatin, cis-diaminedichloroplatinum(II), prompted researchers’ curiosity in the application of metal complexes for cancer. Rosenberg in 1969 [4] serendipitously discovered, cisplatin and was approved in 1978 for the cure of solid cancers worldwide [5]. However, severe adverse effects of cisplatin and its analogs [6–9] include nephrotoxicity, peripheral neuropathy, asthenia, myelosuppression, and ototoxicity. These severe intrinsic and acquired drug confrontations during medication have further restricted the use of cisplatin to a significant extent [10]. As a result, boosted interest in the medicinal chemist fraternity to look for more biocompatible metal ions. Numerous metal-based potential drug candidates have been designed and evaluated for their applications in the field of biomedical [11–13].

In fine-tuning of the cytotoxic potential of the metal-based drug candidate, the role of ligands has considerable importance. Ligands play a significant role in governing the reactivity, the oral/systemic bioavailability of metal ions, oxidation state stabilization, and inertness for substitution, liable on the desires for chemotherapeutics. In ligand motifs, Schiff bases or azomethines are one of the most important groups of biomolecules and have shown to possess significant biological activities [14–16]. For the designing of cytotoxic and cytostatic agents, the assessment of novel synthetic Schiff bases has irrefutably been growing exponentially because of their proven effectiveness as attractive frontrunner structures. An enormous number of the Schiff base compounds have exhibited therapeutic potential. They have shown remarkable biological activities, including anti-tumor, anti-bacterial, anti-malarial, anti-viral, anti-oxidant, anti-fungal, anti-inflammatory, analgesic, anti-convulsant, anti-glycation, anti-hypertensive, anti-depressant and lipid-lowering properties ([14–16] and Ref. therein).

In the field of research in chemical biology and pharmacology, the interaction between drug candidates and plasma proteins is quite exciting [17]. In the human blood, the most abundant protein found is human serum albumin (HSA). It acts as a transporter and dispose of various endogenous and exogenous substances via the circulation and binding to human serum albumin in plasma [18]. The binding of a drug to albumin can lead to increasing solubility of drug, decreasing drugs toxicity, and protects drug from oxidation. Moreover, HSA gets accumulated in tumor cell [19], and taken up at increased levels by tumor cells in comparison to healthy cells. Hence, for various anticancer drugs functions as carrier conjugate e.g. chlorambucil, paclitaxel, and doxorubicin [20,21]. Hence, to study the binding of drugs to the protein is...
Countering drug resistance, infectious diseases, and sepsis using metal and metal oxides nanoparticles: Current status

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A B S T R A C T
One fourth of the global mortalities is still caused by microbial infections largely due to the development of resistance against conventional antibiotics among pathogens, the resurgence of old infectious diseases and the emergence of hundreds of new infectious diseases. The lack of funds and resources for the discovery of new antibiotics necessitates the search for economic and effective alternative antimicrobial agents. Metal and metal oxide nanoparticles including silver and zinc oxide exhibit remarkable antimicrobial activities against pathogens and hence are one of the most propitious alternative antimicrobial agents. These engineered nanomaterials are approved by regulatory agencies such as USFDA and Korea’s FITI, for use as antimicrobial agents, supplementary antimicrobials, food packaging, skin care products, oral hygiene, and for fortifying devices prone to microbial infections. Nevertheless, detailed studies on molecular and biochemical mechanisms underlying their antimicrobial activity are missing. To take the full advantage of this emerging technology selective antimicrobial activity of these nanoparticles against pathogens should be studied. Optimization of these nanomaterials through functionalization to increase their efficacy and biocompatibility is also required. Urgent in vivo studies on the toxicity of nanomaterials at realistic doses are also needed before their clinical translation.

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Contents
1. Introduction ........................................... 71
2. Antibiotic resistance in some crucial pathogens ...................................................... 71
3. Fighting infectious diseases and antibiotic resistance is expensive ................. 72
4. Alternatives to conventional antibiotics ............................................................. 73
5. Combating antibiotic resistance with nanoantibiotics ........................................ 74
5.1. Silver nanoparticles (Ag NPs) ................................................................. 74
5.2. Zinc oxide nanoparticles (ZnO NPs) .......................................................... 74
5.3. Copper oxide nanoparticles (CuO NPs) ....................................................... 75
5.4. Titanium dioxide nanoparticles (TiO₂ NPs) ................................................ 75
5.5. Other nanoparticles ....................................................................................... 76
6. Selective antimicrobial activity of nanoparticles ............................................. 76
7. Commercial products containing metal and metal oxide NPs ......................... 77
7.1. Applications of nanoantibiotics in prevention of infectious diseases ............ 77
7.2. Antimicrobial surfaces and controlling sepsis ............................................. 77
7.3. Biomedical implants .................................................................................... 77
7.4. Food packaging ............................................................................................ 78

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Verbesina encelioides: cytotoxicity, cell cycle arrest, and oxidative DNA damage in human liver cancer (HepG2) cell line

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Abstract

Background: Cancer is a major health problem and exploiting natural products have been one of the most successful methods to combat this disease. Verbesina encelioides is a notorious weed with various pharmacological properties. The aim of the present investigation was to screen the anticancer potential of V. encelioides extract against human lung cancer (A-549), breast cancer (MCF-7), and liver cancer (HepG2) cell lines.

Methods: A-549, MCF-7, and HepG2 cells were exposed to various concentrations of (10–1000 μg/ml) of V. encelioides for 24 h. Further, cytotoxic concentrations (250, 500, and 1000 μg/ml) of V. encelioides induced oxidative stress (GSH and LPO), reactive oxygen species (ROS) generation, mitochondrial membrane potential (MMP), cell cycle arrest, and DNA damage in HepG2 cells were studied.

Results: The exposure of cells to 10–1000 μg/ml of extract for 24 h, revealed the concentrations 250–1000 μg/ml was cytotoxic against MCF-7 and HepG2 cells, but not against A-549 cells. Moreover, the extract showed higher decrease in the cell viability against HepG2 cells than MCF-7 cells. Therefore, HepG2 cells were selected for further studies viz. oxidative stress (GSH and LPO), reactive oxygen species (ROS) generation, mitochondrial membrane potential (MMP), cell cycle arrest, and DNA damage. The results revealed differential anticancer activity of V. encelioides against A-549, MCF-7 and HepG2 cells. A significant induction of oxidative stress, ROS generation, and MMP levels was observed in HepG2 cells. The cell cycle analysis and comet assay showed that V. encelioides significantly induced G2/M arrests and DNA damage.

Conclusion: These results indicate that V. encelioides possess substantial cytotoxic potential and may warrant further investigation to develop potential anticancer agent.

Keywords: Verbesina encelioides, Cytotoxicity, Oxidative stress, ROS generation, MMP, DNA damage

Background

Cancer is one of the main causes of human death in developed and developing countries [1–3]. 15 million new cases of cancer are expected, 70 % of which will be in developing countries by 2020, where governments are less prepared to address the growing cancer burden [4]. Lung cancer, breast cancer, and liver cancer were the most common sites of cancer diagnosed in 2012 among men and women [5]. The growing trend indicates deficiency in the present cancer therapies and the average survival rates are less [6]. An accurate and effective treatment of the cancer is very much required for diagnosis of the specific type of cancer diseases. Every cancer type requires a specific course of therapy that includes one or more modalities such as surgery, and/or radiotherapy, and/or chemotherapy [7, 8]. Therefore, there is an urgent need to explore anti-cancer drugs with higher efficacy with less side effects and an affordable cost [9]. Chemotherapy is...
Cobalt oxide nanoparticles aggravate DNA damage and cell death in eggplant via mitochondrial swelling and NO signaling pathway

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Abstract

Background: Despite manifold benefits of nanoparticles (NPs), less information on the risks of NPs to human health and environment has been studied. Cobalt oxide nanoparticles (Co3O4-NPs) have been reported to cause toxicity in several organisms. In this study, we have investigated the role of Co3O4-NPs in inducing phytotoxicity, cellular DNA damage and apoptosis in eggplant (Solanum melongena L. cv. Violetta lunga 2). To the best of our knowledge, this is the first report on Co3O4-NPs showing phytotoxicity in eggplant.

Results: The data revealed that eggplant seeds treated with Co3O4-NPs for 2 h at a concentration of 1.0 mg/ml retarded root length by 81.5% upon 7 days incubation in a moist chamber. Ultrastructural analysis by transmission electron microscopy (TEM) demonstrated the uptake and translocation of Co3O4-NPs into the cytoplasm. Intracellular presence of Co3O4-NPs triggered subcellular changes such as degeneration of mitochondrial cristae, abundance of peroxisomes and excessive vacuolization. Flow cytometric analysis of Co3O4-NPs (1.0 mg/ml) treated root protoplasts revealed 157, 282 and 178% increase in reactive oxygen species (ROS), membrane potential (ΔΨm) and nitric oxide (NO), respectively. Besides, the esterase activity in treated protoplasts was also found compromised. About 2.4-fold greater level of DNA damage, as compared to untreated control was observed in Comet assay, and 73.2% of Co3O4-NPs treated cells appeared apoptotic in flow cytometry based cell cycle analysis.

Conclusion: This study demonstrate the phytotoxic potential of Co3O4-NPs in terms of reduction in seed germination, root growth, greater level of DNA and mitochondrial damage, oxidative stress and cell death in eggplant. The data generated from this study will provide a strong background to draw attention on Co3O4-NPs environmental hazards to vegetable crops.

Keywords: Cobalt oxide nanoparticles, Nanotoxicity, DNA damage, Apoptosis, Oxidative stress

Background

Over a last decade, nanotechnology has gained an immense research interest due to its applications in public health, medicine, industry and agriculture. The incessant use of nanoparticles (NPs) in a multitude of sectors presents a risk of their release into the environment, which may pose serious threats on ecosystem and adversely affect its living entity [1]. Particularly, plants are at maximum risk due to the concentration build-up of NPs in natural sediments, agricultural soils, and aquatic environments [1, 2]. Recent evidences on the NPs toxicity demonstrated the cellular uptake of Ag-NPs in Oryza Sativa and Cu/CuO-NPs in Lactuca sativa [3, 4]. Vicia
Differential cytotoxicity of copper ferrite nanoparticles in different human cells

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Abstract: Copper ferrite nanoparticles (NPs) have the potential to be applied in biomedical fields such as cell labeling and hyperthermia. However, there is a lack of information concerning the toxicity of copper ferrite NPs. We explored the cytotoxic potential of copper ferrite NPs in human lung (A549) and liver (HepG2) cells. Copper ferrite NPs were crystalline and almost spherically shaped with an average diameter of 35 nm. Copper ferrite NPs induced dose-dependent cytotoxicity in both types of cells, evident by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and neutral red uptake assays. However, we observed a quite different susceptibility in the two kinds of cells regarding toxicity of copper ferrite NPs. Particularly, A549 cells showed higher susceptibility against copper ferrite NP exposure than those of HepG2 cells. Loss of mitochondrial membrane potential due to copper ferrite NP exposure was observed. The mRNA level as well as activity of caspase-3 enzyme was higher in cells exposed to copper ferrite NPs. Cellular redox status was disturbed as indicated by induction of reactive oxygen species (oxidant) generation and depletion of the glutathione (antioxidant) level. Moreover, cytotoxicity induced by copper ferrite NPs was efficiently prevented by N-acetylcysteine treatment, which suggests that reactive oxygen species generation might be one of the possible mechanisms of cytotoxicity caused by copper ferrite NPs. To the best of our knowledge, this is the first report showing the cytotoxic potential of copper ferrite NPs in human cells. This study warrants further investigation to explore the mechanisms of differential toxicity of copper ferrite NPs in different types of cells. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: copper ferrite NPs; cytotoxicity; A549 cells; HepG2 cells; Oxidative stress

Introduction

Nanotechnology has now been recognized as a promising field that provides opportunity for the development of nanostructured materials (1–100 nm size) with unique physicochemical properties. Magnetic nanoparticles (NPs) are used in biomedical applications because of their interesting properties such as superparamagnetic behavior, high surface-to-volume ratio and external magnetic force (Al-Qubaisi et al., 2013; Sahoo et al., 2014). Spinel ferrite \( X\text{Fe}_2\text{O}_4 \) (where \( X = \text{Cu}, \text{Ni}, \text{Zn}, \text{Mg}, \text{Co}, \text{etc.} \)) NPs are a very important class of magnetic materials because of their unique optical, electronic and magnetic properties. Spinel ferrite NPs have a high permeability, good saturation magnetization and no preferred direction of magnetization (Sun et al., 2008). They are magnetically soft, being easily magnetized and demagnetized, and electrically insulating. Copper ferrite NP is one of the important spinel ferrites because it exhibits phase transition, changes semiconducting property, shows electrical switching and tetragonality variation under various situations in addition to interesting magnetic and electrical properties with great thermal and chemical stabilities (Rashad et al., 2012; Sartale et al., 2003). It is utilized in the wide range of applications, including gas sensing, catalytic applications, Li ion batteries, high density magneto-optic recording devices, color imaging, bioprocessing, magnetic refrigeration and ferrofluids (Rashad et al., 2012; Roy and Ghose, 2006; Sun et al., 2007). Copper ferrite NPs also possess a great potential for their application in biomedical fields such as diagnostic imaging, cell labeling, site-directed drug delivery and hyperthermia. However, using copper ferrite NPs for biomedical purposes remains a challenge due to lack of our understanding on the biological response of these NPs at the cellular and molecular level.

The toxicity assessment of spinel ferrite NPs is gaining momentum. Saquib et al. (2013 suggested that zinc ferrite (ZnFe\(_2\)O\(_4\)) NPs trigger apoptosis and/or necrosis in human amnion epithelial (WISH) cells through the mitochondria-dependent intrinsic apoptotic pathway. Horev-Azaria et al. (2013 reported that copper ferrite NPs induced toxicity to different cell lines (e.g., A549, HepG2, NCIH441, MDCK, L929).

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Genotoxicity of ferric oxide nanoparticles in *Raphanus sativus*: Deciphering the role of signaling factors, oxidative stress and cell death

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ABSTRACT

We have studied the genotoxic and apoptotic potential of ferric oxide nanoparticles (Fe2O3-NPs) in *Raphanus sativus* (radish). Fe2O3-NPs retarded the root length and seed germination in radish. Ultrathin sections of treated roots showed subcellular localization of Fe2O3-NPs, along with the appearance of damaged mitochondria and excessive vacuolization. Flow cytometric analysis of Fe2O3-NPs (1.0 mg/mL) treated groups exhibited 219.5%, 161%, 120.4% and 161.4% increase in intracellular reactive oxygen species (ROS), mitochondrial membrane potential (ΔΨm), nitric oxide (NO) and Ca2+ influx in radish protoplasts. A concentration dependent increase in the antioxidative enzymes glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and lipid peroxidation (LPO) has been recorded. Comet assay showed a concentration dependent increase in deoxyribonucleic acid (DNA) strand breaks in Fe2O3-NPs treated groups. Cell cycle analysis revealed 88.4% of cells in sub-G1 apoptotic phase, suggesting cell death in Fe2O3-NPs (2.0 mg/mL) treated group. Taking together, the genotoxicity induced by Fe2O3-NPs highlights the importance of environmental risk associated with improper disposal of nanoparticles (NPs) and radish can serve as a good indicator for measuring the phytotoxicity of NPs grown in NP-polluted environment.

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Zinc oxide Quantum Dots: Multifunctional candidates for arresting the C2C12 cancer cells and their role towards Caspase 3 and 7 genes

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Recently, nanoscale (<100 nm) inorganic materials, especially spherical shaped zinc oxide (ZnO-QDs) have received significant attention from broad community because of their potential utilizations in various technologies. Due to their large surface to volume (S/V) ratio contributions and extremely high reactivities, they can easily penetrate in various biological identities, like cells, proteins and hence can sense, diagnose and cure different biological systems. The present study describes the simple synthesis of crystalline ZnO-QDs via solution process and C2C12 myoblast cancer cells have been treated with different doses of ZnO-QDs at different incubation (24, 48, 72 and 96 h) time. The rate of inhibition of cells was observed with MTT assay whereas morphology of cells was observed via confocal microscopy (CLSM). The MTT and CLSM investigations confirmed that with increase in the incubation time, the population density of cancer cells was decreased when treated with ZnO-QDs. The dose dependent apoptosis correlated intracellular production of reactive oxygen species (ROS) from C2C12 cancer cells was also measured in presence of ZnO-QDs. Apart from this, the effect/apoptosis of these QDs were also checked in presence of candidate genes such as caspase 3/7 with GAPDH. The Reverse transcription polymerase chain reaction (RT-PCR) analysis demonstrates the up-regulation of caspase 3/7 genes in cells subsequently treated with ZnO-QDs at low and high concentrations.

Introduction

Cancer disease is currently a very major issue in the society because of several deaths and the biomedical society is still looking for an appropriate alternative, which can kill cancer cells but at the same time it must be biocompatible so that normal and healthy cells remain unaffected. Recent developments in nanotechnology have provided new platforms of different kinds of nanostructures, which can be effectively used in biomedical engineering for various treatments.

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Owing to their S/V ratio in nanoscale region, these nanostructures exhibit extraordinary physical and chemical features, which enable them as potential building blocks for several multifunctional applications in different disciplines and particularly in biomedical field. For example very small nanostructures (~1-100 nm) can be effectively utilized in identify and cure of diverse categories of cancers\textsuperscript{1} Different nanostructures find potential role for the detection of DNA, intracellular labeling, drug delivery, blocking viral entry into the cells, cancer targeting and imaging etc.\textsuperscript{2-11} Also they are heavily utilized in cell and molecular biology, as markers and probes, for tissue engineering, for clinical bio-analytical diagnostics and therapeutics.\textsuperscript{11-12} Nanostructures of metal oxides are very particular which are significantly used by industries for making several products in the market for day today life, e.g., cosmetics, catalysts, fillers and drug carriers.\textsuperscript{13} Among various metal oxides, zinc oxide nanostructures has drawn special attention for the materials scientist due to its bio compatible nature, low cost, easy to process and has already shown potentials applications in various fields such as sun screens, photocatalysis, cosmetic products, optoelectronic devices, etc.\textsuperscript{14-16} In this regards, ZnO nanostructures, which have very small size (diameter <10 nm), are known as quantum dots (QDs), and are particularly very important because of their very high S/V ratio and
Zinc oxide and titanium dioxide nanoparticles induce oxidative stress, inhibit growth, and attenuate biofilm formation activity of Streptococcus mitis

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Abstract Streptococcus mitis from the oral cavity causes endocarditis and other systemic infections. Rising resistance against traditional antibiotics amongst oral bacteria further aggravates the problem. Therefore, antimicrobial and antibiofilm activities of zinc oxide and titanium dioxide nanoparticles (NPs) synthesized and characterized during this study against S. mitis ATCC 6249 and Ora-20 were evaluated in search of alternative antimicrobial agents. ZnO and TiO2-NPs exhibited an average size of 35 and 13 nm, respectively. The IC50 values of ZnO and TiO2-NPs against S. mitis ATCC 6249 were 37 and 77 µg ml−1, respectively, while the IC50 values against S. mitis Ora-20 isolate were 31 and 53 µg ml−1, respectively. Live and dead staining, biofilm formation on the surface of polystyrene plates, and extracellular polysaccharide production show the same pattern. Exposure to these nanoparticles also shows an increase (26–83 %) in super oxide dismutase (SOD) activity. Three genes, namely bapA1, sodA, and gtfB like genes from these bacteria were identified and sequenced for quantitative real-time PCR analysis. An increase in sodA gene (1.4- to 2.4-folds) levels and a decrease in gtfB gene (0.5- to 0.9-folds) levels in both bacteria following exposure to ZnO and TiO2-NPs were observed. Results presented in this study verify that ZnO-NPs and TiO2-NPs can control the growth and biofilm formation activities of these strains at very low concentration and hence can be used as alternative antimicrobial agents for oral hygiene.

Keywords ZnO · TiO2 · Nanoparticles · Oral hygiene · Alternative antimicrobials · S. mitis

Introduction

Oral bacteria pose a serious health challenge as an etiological agent of several systemic infections besides dental and periodontal diseases [1]. Emerging antibiotic resistance among these bacteria further aggravates the problem of oral hygiene and of systemic diseases resulting from recurring oral infections [2]. Infections of Streptococcus mitis, a commensal oral bacterium, and a low virulence pathogen lead to endocarditis, sepsis in neutropenic patients, toxic shock like syndrome, and pancreatic cancer [3–6]. Moreover, the incidence of multidrug resistance in S. mitis including resistance to β-lactams, tetracycline, and aminoglycosides is a matter of concern [7, 8]. Whole genome sequence and other studies also confirm the presence of genetic information for multidrug resistance in S. mitis [9]. Therefore, it is of immense importance to minimize the population of S. mitis in the oral cavity and/or to control other systemic infections caused by this bacterium using alternative antimicrobial agents.
Self-Styled ZnO Nanostructures Promotes the Cancer Cell Damage and Supresses the Epithelial Phenotype of Glioblastoma

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Extensive researches have been done on the applications of zinc oxide nanoparticles (ZnO-NPs) for the biological purposes. However, the role and toxicity mechanisms of ZnO nanostructures (ZnO-NSts) such as nanoplates (NPls), nanorods (NRs), nanosheets (NSs), nanoflowers (NFs) on cancer cells are not largely known. Present study was focused to investigate the possible mechanisms of apoptosis induced by self-designed ZnO-NSts, prepared at fix pH via solution process and exposed against human T98G gliomas including various cancers and non-malignant embryonic kidney HEK293, MRC5 fibroblast cells. NSts were used for the induction of cell death in malignant human T98G gliomas including various cancers and compared with the non-malignant cells. Notably, NRs were found to induce higher cytotoxicity, inhibitory effects on cancer and normal cells in a dose dependent manner. We also showed that NRs induced cancer cell death through oxidative stress and caspase-dependent pathways. Furthermore, quantitative and qualitative analysis of ZnO-NSts have also been confirmed by statistical analytical parameters such as precision, accuracy, linearity, limits of detection and limit of quantitation. These self-styled NSts could provide new perception in the research of targeted cancer nanotechnology and have potentiality to improve new therapeutic outcomes with poor diagnosis.

Over the past decade the use of inorganic metal oxides (MOs) semiconductor based nano- materials has gained interest very rapidly in the area of electronic, industries and biomedical field1–4. These materials have special attention due to their very small size, high surface area, and inexpensive as compared to the organic materials5. Among various semiconductor materials, the MOs of ZnO nanostructures (NSts), which exhibit wider range of NSts such as nanoplates (NPls), nanorods (NRs), nanosheets (NSs), nanoflowers (NFs) etc has special place with large applications in various optoelectronics areas for instance photooxidation, photocatalysis, solar cells, light emitting (LED), sunscreen, piezoelectric. These materials are also applied for sensors, cosmetic products, clothing, paints, and various biological systems6–12. These material has easy preparation process, which itself makes prominent, cost effective and gives various types of NSts6. The ZnO-NSts can be prepared via various processes such as thermal, aqueous and non-aqueous processes as described in previous reports10,13,14. Currently ZnO-NSts have been focused for various biological applications due to their biocompatible nature6. In the area of biological applications, there are enough quantity of research have been published towards the application of NPs and their role to control cancer cells growth but mechanism of cytotoxicity caused through ZnO-NSts has remained obscure15–17. Accumulating evidences suggested the reasons of cytotoxicity of ZnO-NSts through reactive oxygen species (ROS) and genotoxicity in cancer cells15. A recent report showed that the toxicity of cancer cells happens due to release of Zn2+ ions in zinc oxide solution18. Sharma et al. reported that the nanoscale zinc oxide induced DNA damage through lipid peroxidation and oxidative stress in human epidermal cells19. Among various types

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Antibacterial studies and statistical design set data of quasi zinc oxide nanostructures†

Rizwan Wahab,ab Farheen Khan, c Yogendra Kumar Mishra, d Javed Musarrat ef and Abdulaziz A. Al-Khedhairya

The present paper describes a systematic study of bacterial growth measured and analysed via UV-visible spectroscopy, which reveals a strong dependence on pH. The morphology of the zinc oxide (ZnO) nanostructures (from sheets to flowers) varies with respect to change in pH and hence their different abilities to inhibit the bacterial (E. coli, S. aureus and K. pneumoniae) densities. The solution of zinc acetate dihydrate (Zn(CH3COO)2·2H2O) was optimized by the addition of NaOH and HCl to obtain various pH values, ranging from pH 7 to 12. The optimized (pH 7, 10 and 12) solutions of zinc acetate dihydrate were further refluxed to obtain the different morphologies, and subsequently qualitative and quantitative determinations were studied. Absorption spectra of the resulting solutions were recorded at desired pH values, and all measurements were obtained at 600 nm with respect to corresponding control solution or blank. The linearity of the proposed method was evaluated at five concentration levels in the range from 0.5 to 2.0 μg mL⁻¹. Minute quantities of the different morphological nanostructures were used to determine the analytical parameters, such as correlation coefficient (r² = 0.9995, 0.9998, 0.9990), limit of detection (LOD, 0.053, 0.027 and 0.072 μg mL⁻¹), limit of quantitation (LOQ, 0.016, 0.083 and 0.220 μg mL⁻¹), respectively. Relative standard deviation and quantitative recoveries (RSD%) range from 0.113 to 1.58% and 98.66–100.88%. The morphologies of bacteria (E. coli, S. aureus and K. pneumoniae) and their interactions with synthesized ZnO nanostructures were analysed with Bio-TEM. The study suggests that the grown ZnO nanostructures with variable morphologies exhibit good accuracy and precision, revealed by statistical parameters and recovery data.

1. Introduction

The controlled growth of zinc oxide (ZnO) nanomaterials and their corresponding detailed mechanisms explain several observed morphologies, including nanoparticles,1–3 nanorods,4 nanowires,5 nanobelts,6 nanonuts,7 nanotubes,8 nanorings,9 nanobridges,10 nanonails,11 nanocubes,12 nanosheets,13 sea urchin shapes,14 nanoflowers,15,16 Solution-based syntheses of all these nanostructures are highly influenced by pH.13–16 In particular, the effect of pH on ZnO nanostructures plays an important role in determining their antibacterial properties; structures with nanoporous surfaces easily penetrate cell membranes exhibited by bacteria and gradually destroy cell walls.13–17 Recently, the most investigated applications of ZnO nanostructures in electronics are lasers, transistors, photo detectors, sensors, piezoelectric, photovoltaics, optoelectronic, and photocatalysts.6,18–19 Several fabrication methods have been employed for formation of ZnO nanostructures, such as thermal evaporation (TE), hydrothermal growth (HG), metal–organic chemical vapour deposition (MOCVD), ion beam assisted deposition (IBAD), laser-ablation (LA), sputter deposition (SD), electro chemical deposition (ECD), sol–gel growth, aqueous and non-aqueous methods.20–28 According to previous reports, the ZnO nanostructures manufactured within the pH range have received considerable attention because final features exhibit physicochemical properties, such as high surface area, non-toxicity, thermal, chemical, electrochemical, and high mechanical strength.29–31 Due to its biocompatibility with human cells, ZnO has been widely used for antibacterial purposes under normal UV conditions because it exhibited superior durability, high selectivity towards, heat resistance, and chemical stability.32

Over the past decade, several reports have published the effects of pH precursor solution (as buffer solution) and reagents on growth of ZnO nanostructures and synthesized...
Hazards of low dose flame-retardants (BDE-47 and BDE-32): Influence on transcriptome regulation and cell death in human liver cells

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HIGHLIGHTS

• First report on low dose molecular toxicity of BDE-47 and BDE-32.
• Both congeners induce DNA damage and mitochondrial dysfunction in HepG2 cells.
• Transcriptomic alterations were found in BDE-47 and BDE-32 treated cells.
• BDE-47 and BDE-32 exposure trigger apoptosis in HepG2 cells.

ABSTRACT

We have evaluated the in vitro low dose hepatotoxic effects of two flame-retardants (BDE-47 and BDE-32) in HepG2 cells. Both congeners declined the viability of cells in MTT and NRU cell viability assays. Higher level of intracellular reactive oxygen species (ROS) and dysfunction of mitochondrial membrane potential (ΔΨm) were observed in the treated cells. Comet assay data confirmed the DNA damaging potential of both congeners. BDE-47 exposure results in the appearance of subG1 apoptotic peak (30.1%) at 100 nM, while BDE-32 arrested the cells in G2/M phase. Among the set of 84 genes, BDE-47 induces downregulation of majority of mRNA transcripts, whilst BDE-32 showed differential expression of transcripts in HepG2. The ultrastructural analysis revealed mitochondrial swelling and degeneration of cristae in BDE-47 and BDE-32 treated cells. Overall our data demonstrated the hepatotoxic potential of both congeners via alteration of vital cellular pathways.

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Cobalt oxide nanoparticles aggravate DNA damage and cell death in eggplant via mitochondrial swelling and NO signaling pathway

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Abstract

Background: Despite manifold benefits of nanoparticles (NPs), less information on the risks of NPs to human health and environment has been studied. Cobalt oxide nanoparticles (Co3O4-NPs) have been reported to cause toxicity in several organisms. In this study, we have investigated the role of Co3O4-NPs in inducing phytotoxicity, cellular DNA damage and apoptosis in eggplant (Solanum melongena L. cv. Violetta lunga 2). To the best of our knowledge, this is the first report on Co3O4-NPs showing phytotoxicity in eggplant.

Results: The data revealed that eggplant seeds treated with Co3O4-NPs for 2 h at a concentration of 1.0 mg/ml retarded root length by 81.5 % upon 7 days incubation in a moist chamber. Ultrastructural analysis by transmission electron microscopy (TEM) demonstrated the uptake and translocation of Co3O4-NPs into the cytoplasm. Intracellular presence of Co3O4-NPs triggered subcellular changes such as degeneration of mitochondrial cristae, abundance of peroxisomes and excessive vacuolization. Flow cytometric analysis of Co3O4-NPs (1.0 mg/ml) treated root protoplasts revealed 157, 282 and 178 % increase in reactive oxygen species (ROS), membrane potential (ΔΨm) and nitric oxide (NO), respectively. Besides, the esterase activity in treated protoplasts was also found compromised. About 2.4-fold greater level of DNA damage, as compared to untreated control was observed in Comet assay, and 73.2 % of Co3O4-NPs treated cells appeared apoptotic in flow cytometry based cell cycle analysis.

Conclusion: This study demonstrate the phytotoxic potential of Co3O4-NPs in terms of reduction in seed germination, root growth, greater level of DNA and mitochondrial damage, oxidative stress and cell death in eggplant. The data generated from this study will provide a strong background to draw attention on Co3O4-NPs environmental hazards to vegetable crops.

Keywords: Cobalt oxide nanoparticles, Nanotoxicity, DNA damage, Apoptosis, Oxidative stress

Background

Over a last decade, nanotechnology has gained an immense research interest due to its applications in public health, medicine, industry and agriculture. The incessant use of nanoparticles (NPs) in a multitude of sectors presents a risk of their release into the environment, which may pose serious threats on ecosystem and adversely affect its living entity [1]. Particularly, plants are at maximum risk due to the concentration build-up of NPs in natural sediments, agricultural soils, and aquatic environments [1, 2]. Recent evidences on the NPs toxicity demonstrated the cellular uptake of Ag-NPs in Oryza sativa and Cu/CuO-NPs in Lactuca sativa [3, 4]. Vicia
In-Vitro dual inhibition of protein glycation, and oxidation by some Arabian plants

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Abstract

Background: Diabetes mellitus is a metabolic disorder of epidemic proportion, projected to become the major cause of morbidity and mortality in the world in future. Despite extensive research in understanding this disease at molecular level, and the discovery of new drugs, diabetes and its complications remain largely untreated. Many of the late diabetic complications are associated with the glycation of proteins in the body. Natural flora has long been a rich source for therapeutic agents, especially against diabetes. The present study deals with the anti-glycation properties of some medicinally important plants of Arabian region.

Methods: Twenty-six medicinal plants, commonly found in different regions of Arabian Peninsula, were evaluated for their protein anti-glycation activity by using BSA-MG glycation assay in-vitro. The extracts were incubated with BSA and MG at 37 °C for 9 days, each sample was then examined for the presence of fluorescence (λex 330 nm, λem 420 nm), which represent the extent of protein glycation. Antioxidant activity was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), iron chelation, and superoxide radical scavenging assays.

Results: The data revealed that out of 26 medicinal plants, five plants viz. Sida cordifolia, Plumbago zeylanica, Tribulus terrestris, Glycyrrhiza glabra, and Rosa indica were active against the in-vitro protein glycation with IC50 values between 0.408-1.690 mg/mL. Among the active plants, Glycyrrhiza glabra L. was found to be the most potent (IC50 = 0.408±0.027 mg/mL), followed by Rosa indica (IC50 = 0.596±0.0179 mg/mL), and Sida cordifolia L. (IC50 = 0.63±0.009 mg/mL). The antioxidant potential of these plant extracts were also determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl), iron chelation, and superoxide anion radical scavenging assays. Among five plants, Sida cordifolia exhibited a potent anti-oxidant activity in both DPPH and superoxide anion radical scavenging assays (IC50 = 0.005±0.0004, and 0.078±0.002 mg/mL, respectively), followed by Rosa indica (IC50 = 0.023±0.0005 and 0.141±0.003 mg/mL, respectively).

Conclusions: Protein glycation in hyperglycemic conditions involve oxidative changes. Therefore dual inhibition of protein glycation and oxidation are desirable properties in any test substance investigated for therapeutic purposes.

Keywords: Arabian medicinal plants, Diabetes, Advanced glycation end products (AGEs), Antioxidant, Glycyrrhiza glabra L., Rosa indica L., Sida cordifolia L.

Background

Diabetes mellitus (DM) is an impending public health challenge of the present century [1]. It affects over 387 million people globally, and this number is projected to increase to 592 million by 2035. DM is currently the fourth leading cause of mortality in the world. It has also emerged as a major socioeconomic burden for developing countries [2]. In last three decades, extensive research has been conducted on glycation and anti-glycation processes in diabetes, based on the fact that the hyperglycemic condition or excess glucose in blood leads to the binding of free sugars with biomolecules [3–5]. Glycation is a spontaneous, non-enzymatic reaction between biomolecules (proteins, lipids, and DNA) and reducing sugars (such as glucose, fructose, and ribose), resulting in the formation of advanced glycation endproducts (AGEs) [6–8]. The accelerated process of proteins glycation has been identified as a
Portulaca oleracea Linn seed extract ameliorates hydrogen peroxide-induced cell death in human liver cells by inhibiting reactive oxygen species generation and oxidative stress

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Abstract

Purpose: To investigate the protective effects of Portulaca oleracea seed extract (POA) against cytotoxicity, oxidative stress and reactive oxygen species (ROS) generation induced by hydrogen peroxide (H₂O₂) in human liver cells (HepG2).

Methods: The extract (POA) was obtained by ethanol extraction of P. oleracea seeds. Cytotoxicity in HepG2 cells was assessed by 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide (MTT) assay, neutral red uptake (NRU) assay and morphological changes. The cells were pre-exposed to non-cytotoxic concentrations (5-25 µg/mL) of POA for 24 h, and then cytotoxic (0.25 mM) concentration of H₂O₂. After 24 h of exposure, MTT and NRU assays were used to evaluate cell viability, while morphological changes were assessed using phase contrast inverted microscopy. The effect of POA on reduced glutathione (GSH) level, lipid peroxidation (LPO), and ROS generation induced by H₂O₂ was also studied.

Results: The results showed that pre-exposure to POA (25 µg/mL) significantly (p <0.01) attenuated the loss of cell viability by up to 38 % against H₂O₂-induced oxidative stress and ROS generation. In addition, POA (25 µg/mL) significantly (p <0.01) increased GSH level (31 %), but decreased the levels of LPO (37 %) and ROS generation (49 %).

Conclusion: This study demonstrates that POA has the capacity to protect HepG2 cells against H₂O₂-induced cell death by inhibiting oxidative stress and ROS generation.

Keywords: Portulaca oleracea, HepG2 cells, Cytotoxicity, Oxidative stress, Reactive oxygen species

INTRODUCTION

Oxidative stress is caused by an imbalance in the amount of reactive oxygen species (ROS) and antioxidant defense systems in biological system [1]. It is one of the most important factors inducing cell apoptosis [2]. Oxidative stress can increase the vulnerability to lipid peroxidation, DNA damage, enzymatic inactivation, and cell death [3]. It has been reported that overproduction of ROS plays a major role in hepatocarcinoma [4], and cellular damage [5]. H₂O₂ has been reported to induce cytotoxicity and apoptotic cell death in a variety of cell
Antimutagenic Potential of *Laurus nobilis* L. in *Salmonella typhimurium* Test System

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**Abstract** - Leaf extract of *laurus nobilis* L. was prepared in methanol and concentrated to dryness. Different concentration of the extract was tested for their antimutagenic activity using *Salmonella typhimurium* test assay. The tested concentrations of the extract showed no mutagenicity compared to standard mutagens tested (Sodium azide, NA, and Methyl methane sulphonate, MMS). The extract exhibited concentration dependent antimutagenicity. At the highest tested concentration (100 μg/plate), the methanolic extract exhibited the percent inhibition of His+ revertants from 54.22 to 75.76 against sodium azide induced mutagenicity in one or other test strains. Similarly, percent inhibition of mutagenicity (60.87 to 77.49) was also recorded against methyl methane sulphonate (MMS) at 100 μg/plate dose in Salmonella tester strains, TA 97a, TA 100, TA 102 and TA 104. However, at lower concentrations (12.5, 25 and 50 μg/plate) of the plant extract, a decrease in antimutagenic activity was recorded. The data obtained clearly indicated the presence of antimutagenic compounds in the crude extract and need further investigation on isolation of active compounds.

**Keywords:** *Laurus nobilis*, leaf extract, Antimutagenic activity, Mutation, *Salmonella typhimurium*.

**Introduction**

Research in the past has indicated that somatic cell mutations play a key role in initiation and development of various ailments including genetic disorders and cancers (Sarac et al., 2015). Numerous synthetic and natural entities are known for their DNA damaging properties which may lead to mutations and are known as mutagens. These mutagens act by modification of DNA, which can occur at single base (point mutation) or as large deletions or rearrangements of DNA (Mortelmans and Zeiger, 2000). Mechanisms involve in mutagenesis is complex, however, many mutagens and carcinogens may act through generation of reactive oxygen species (ROS) (Słoczyńska et al., 2014). Effect of these mutagenic agents can be counterbalance by variety of secondary metabolites present in plants (Horn and Vergas, 2003). These protective agents, also known as antimutagens, are capable of decreasing the frequency of chemically induced mutations and strengthen cell defenses against environmental mutagens (Kaur et al., 2010). Antimutagens of plant origin belong to various phytochemical structural groups such as flavonoids, tannins and other phenolic secondary metabolites (Musarrat et al., 2006). From cancer prevention point of view, various natural products and pure compounds have been screened for their antimutagenicity. Various biological assays are developed and used to screen antimutagenic properties of natural and chemical substances, including plant derived natural products. These assays include different test system like bacterial system (Ames test), yeast assay and also include plant and animal cell model (Musarrat et al., 2006; Słoczyńska et al., 2014). Plant derived antimutagens are considered as relatively nontoxic and also possess complementary biological activities (Aqil et al., 2008). Therefore, continuous effort in search of novel and broad spectrum antimutagen from food and medicinal plants is needed.

*Laurus nobilis* L. is a medicinal plant, commonly known as 'bay laurel' belonging to the *Lauraceae* family. Leaves of *L. nobilis* (Bay leaf, Tejpatta)
Prevalence of antibiotic resistance and virulence factors encoding genes in clinical Staphylococcus aureus isolates in Saudi Arabia


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Background: The present work intended to investigate the carriage of antibiotic resistance and virulence genes in multidrug-resistant Staphylococcus aureus isolated from various clinical specimens.

Methods: A total of fifty S. aureus isolates from blood cultures, wound swabs, urine sample, nasal swabs, and sputum sample were examined for their antibiotic resistance against 20 different antibiotics by means of E-test, M.I.C Evaluator Strips, and disk diffusion methods. Detection of resistance and virulence-encoding genes (mecA, van, fnBPA, and Panton-Valentine Leucocidin (PVL)-encoding genes) was performed by PCR.

Results: In the current study, low number of MRSA isolates has been detected from five different clinical samples (22%, n = 50). In this study, we observed multiple drug resistance in S. aureus isolates from wound swabs; nasal swabs, blood cultures, and urine sample. No vancomycin-resistant genes were detected in all 50 isolates; similarly, no PVL and van genes were detected, while mecA and FnBP genes were detected in low number of isolates.

Conclusion: Although the number of MRSA and fnBPA-positive S. aureus reported in this study is generally low, and despite the absence of PVL and van-encoding genes, the results reported in this study may continue to shed some light on the prevalence of MRSA in Makkah, Saudi Arabia. Further epidemiological surveys are required together with additional infection control measures to limit the spread of MRSA particularly multidrug-resistant strains.

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Sol-gel synthesis of thorn-like ZnO nanoparticles endorsing mechanical stirring effect and their antimicrobial activities: Potential role as nano-antibiotics

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The effect of mechanical stirring on sol-gel synthesis of thorn-like ZnO nanoparticles (ZnO-NPs) and antimicrobial activities is successfully reported in this study. The in-house synthesized nanoparticles were characterized by XRD, SEM, TEM, FTIR, TGA, DSC and UV-visible spectroscopy. The X-Ray Diffraction analysis revealed the wurtzite crystal lattice for ZnO-NPs with no impurities present. The diametric measurements of the synthesized thorn-like ZnO-NPs (morphology assessed by SEM) were well accounted to be less than 50 nm with the help of TEM. Relative decrease in aspect ratio was observed on increasing the agitation speed. The UV-visible spectroscopy showed the absorption peaks of the ZnO-NPs existed in both UVA and UVB region. A hypsochromic shift in λmax was observed when stirring pace was increased from 500 rpm to 2000 rpm. The FTIR spectroscopy showed the absorption bands of the stretching modes of Zn-O between 500 cm\(^{-1}\) to 525 cm\(^{-1}\). The Thermal analysis studies revealed better stability for ZnO-NPs prepared at 2000 rpm (ZnO-2000 rpm). TGA revealed the weight loss between two main temperatures ranges viz. around (90 °C–120 °C) and (240 °C–280 °C). Finally, the effect of ZnO-NPs prepared at different stirring conditions on the growth of Gram-positive (Bacillus subtilis), Gram-negative (Escherichia coli) bacteria and a fungi (Candida albicans) were examined; which showed good antibacterial as well as antifungal properties. These findings introduce a simple, inexpensive process to synthesize ZnO-NPs using conventional methods without the use of sophisticated equipments and its application as a potent nano-antibiotic.

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Broad-spectrum inhibition of AHL-regulated virulence factors and biofilms by sub-inhibitory concentrations of ceftazidime

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Quorum sensing (QS) in bacteria is a density dependent communication system that regulates the expression of genes, including production of virulence factors in many pathogens. The emergence of antibiotic resistance among pathogenic bacteria represents a major threat in both hospitals as well as environmental settings. Interference of quorum sensing (QS)-regulated virulence factors and biofilms is a recognized anti-pathogenic therapy. Safe, stable and effective anti-QS agents are needed to combat diseases caused by multidrug-resistant bacteria. The present study was performed to assess the inhibitory effect of third generation antibiotic ceftazidime against Gram-negative bacterial pathogens. Sub-MICs of ceftazidime demonstrated dose dependent inhibition of QS regulated virulence traits and biofilm formation in various strains of Chromobacterium violaceum (CV12472 and CV026), Pseudomonas aeruginosa (PAO1 and PAF79) and Aeromonas hydrophila (WAF38). β-galactosidase assay revealed ceftazidime inhibited the las and pqs QS systems in P. aeruginosa. Alongside, in vivo studies demonstrated enhanced survival of Caenorhabditis elegans after the treatment with the drug. Molecular docking analysis showed the high binding affinity of ceftazidime which represents its QS inhibitory activity. By highlighting the broad spectrum anti-quorum sensing and biofilm inhibiting activities against 3 different bacterial pathogens, ceftazidime seems a more potent candidate in counteracting the infections caused by drug resistant bacteria.

Introduction

Bacterial phenotypes such as virulence, secondary metabolite production and biofilm maturation are controlled by cell-to-cell communication, a process commonly known as quorum sensing (QS). N-Acylhomoserine lactones (AHLs) are employed as QS signal molecules in many Gram-negative bacteria. AHL mediated QS systems are composed of two components: a signal (AHL) generator (LuxI homologue) and a response regulator (LuxR homologue) which can bind with the AHLS to form AHL-receptor complexes that regulate the transcription of target QS-regulated genes.²

Pseudomonas aeruginosa QS systems comprise two AHL-mediated LasR/I and RhlR/I and one quinolone based PQS system that work in a hierarchical manner.³ The Las, Rhl and PQS systems regulate multiple genes in P. aeruginosa, which include virulence, drug resistance and programmed cell death.⁴ P. aeruginosa secretes a range of virulence factors, such as elastase,⁵ quorum sensing molecule Pseudomonas quinolone signal (PQS),⁶ pyocyanin,⁷ rhamnolipids,⁸ and siderophores (pyoverdine and pyochelin).⁹ It also produces several adhesion factors like exotoxin A, phospholipase C (used for hemolysis), and exoenzyme S.¹⁰

Quorum sensing in bacteria has been considered as an antinfective drug target as it’s inhibition attenuates bacterial virulence and may help to control infections. Quorum sensing interference is achieved either by degrading QS signals or by interrupting the perception of signal molecules by receptor proteins.¹¹ Since the discovery of halogenated furanones as quorum-sensing inhibitors by Givskov et al.,¹² a variety of synthetic and natural agents have been studied for their QS inhibitory potential both in vitro and in vivo.¹³ Azithromycin (AZM), an azalide (a sub-class of macrolide antibiotics), is one such agent that possesses anti-quorum sensing and anti-biofilm activity but does not show significant bactericidal activity.

Page 1 of 5
Antioxidant and antimutagenic potential of *Psidium guajava* leaf extracts

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**Abstract**

Fruits, vegetables and medicinal herbs rich in phenolics antioxidants contribute toward reduced risk of age-related diseases and cancer. In this study, *Psidium guajava* leaf extract was fractionated in various organic solvents viz. petroleum ether, benzene, ethyl acetate, ethanl and methanol and tested for their antioxidant and antimutagenic properties. Methanolic fraction showed maximum antioxidant activity comparable to ascorbic acid and butylated hydroxyl toluene (BHT) as tested by DPPH free radical scavenging, phosphomolybdenum, FRAP (Fe3+ reducing power) and CUPRAC (cupric ions (Cu2+) reducing ability) assays. The fraction was analyzed for antimutagenic activities against sodium azide (NaN3), methylmethane sulfonate (MMS), 2-aminofluorene (2AF) and benzo(a)pyrene (BP) in Ames *Salmonella* tester strains. The methanol extracted fraction at 80 µg/ml concentration inhibited above 70% mutagenicity. Further, phytochemical analysis of methanol fraction that was found to be most active revealed the presence of nine major compounds by gas chromatography–mass spectrometry (GC–MS). This data suggests that guava contains high amount of phenolics responsible for broad-spectrum antimutagenic and antioxidant properties in *vitro* and could be potential candidates to be explored as modern phytomedicine.

**Introduction**

Reactive oxygen species (ROS) are often generated as by-products of biological reactions or from exogenous. These ROS create homeostatic imbalance which generate oxidative stress and cause cell death and tissue injury and have been associated with physiological and pathological conditions such as aging, cancer, rheumatoid arthritis and atherosclerosis, etc. (Barnham et al., 2004; Pardo-Andreu et al., 2006; Riceevans & Burdon, 1993). The health promoting effect of plants as antioxidants is thought to arise from their potential against reactive oxygen/nitrogen species. Antioxidants provide protection to living organisms from damage triggered by uncontrolled production of ROS and associated lipid peroxidation, protein damage and DNA strand breaking (Kavitha Nair et al., 2010). A great number of medicinal plants have been tested for their antioxidant activities and results have shown that raw extracts or isolated pure compounds from them were effective antioxidants (Gu & Weng, 2001; Pyo et al., 2004; Zahin et al., 2010a,b, 2013). Various phytocompounds have been reported to possess antioxidant activity; however, data on antimutagenic/anticancer compound is scanty and search of safe broad spectrum active compounds is needed (Zahin et al., 2010b).

DNA mutation is considered as a key event in cancer development (Ames et al., 1973). It has been shown that lifestyle, diet and smoking are key factor in cancer development; as a matter of fact, around 35% of cancer cases may be associated with diet (Benigni, 2005). The *in vitro* evaluation of antimutagenicity is commonly used as a first stage to identify new potential anticarcinogenic substances.

Plants are the main source of bioactive metabolites with antimutagenic and anticarcinogenic activities (e.g. phenolics, quinones, glucosinolates, allyl sulfides, terpenoids and alkaloids), and several studies have showed a relationship of these activities with antioxidant capacity (Knasmüller et al., 2004; Santos-Cervantes et al., 2007). Since both the mutagens and carcinogens may act through the generation of reactive oxygen species, the discovery and exploration of plant extracts/phytocompounds possessing both antioxidant and antimutagenic properties are of great practical and therapeutic significance. It is presumed that medicinal plants those are good in antioxidant activity could also show antimutagenic activity and such natural products could reduce or inhibit the mutagenic potential of mutagens and/or procarcinogens
Broad Spectrum Antioxidant Properties of 20 Indian Medicinal Plants

Maryam Zahin, Iqbal Ahmad, Iram Shireen, Fohad Mabood Husain, and Farrukh Aqil

ABSTRACT
Antioxidant properties of methanol extracts of 20 plants were studied by four different assays over a range of concentrations (12.5–400 µg mL⁻¹), and compared with standard antioxidants' ascorbic acid and butylated hydroxytoluene. Ascorbic acid–equivalent antioxidant activity of extracts by the phosphomolybdenum method ranged from 392.8 to 2942.7 µmoles g⁻¹ at 400 µg mL⁻¹, whereas DPPH radical scavenging was between 30.9% and 95.2%; extracts also showed strong activity by FRAP and CUPRAC assays. The extracts demonstrated dose-dependent activity and strong correlation between antioxidant activities and total phenolics. Total phenolic content was the highest in Syzygium aromaticum (279 mg GAE g⁻¹) and the lowest in Sesame indicum (28.16 mg GAE g⁻¹).

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Introduction
The most important groups of reactive oxygen species (ROS) include superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide, which are generated as byproducts of a biological reaction or from exogenous factors. In vivo, although some of these ROS play an important role in cell metabolism including energy production, phagocytosis, and intercellular signaling (9), they cause oxidative damage to lipids, proteins, and nucleic acids, resulting in degenerative diseases. Consequently, antioxidant compounds that can neutralize free radicals may play a major role in the prevention of cancer, cataracts, cerebral pathologies, and rheumatoid arthritis (26).

The use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional, and therapeutic value (2). Many Indian medicinal plants have been explored as potential sources of antioxidants (21, 10, 3). Use of antioxidants from dietary sources, including spices, is a promising alternative
Multidrug resistance and transferability of $\text{bla}_{\text{CTX-M}}$ among extended-spectrum $\beta$-lactamase-producing enteric bacteria in biofilm

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ABSTRACT

This study aimed to investigate the occurrence of biofilm-forming extended-spectrum $\beta$-lactamase (ESBL)-producing enteric bacteria in hospital wastewater and to evaluate their antibiotic resistance behaviour and transferability of the plasmid-encoded $\text{bla}_{\text{CTX-M}}$ gene in biofilm. ESBL production was confirmed using the combined disc test and Etest. Amplification of $\text{bla}_{\text{CTX-M}}$ was performed by PCR. Antibiotic susceptibility was evaluated using the disc diffusion assay and broth dilution method. Transfer of $\text{bla}_{\text{CTX-M}}$ in planktonic and biofilm state was performed by broth mating and filter mating experiments, respectively. Among 110 enteric bacteria, 24 (21.8%) isolates belonging to Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae were found to produce ESBL and formed varying levels of biofilm in vitro. Presence of $\text{bla}_{\text{CTX-M}}$ was detected in 18 (75%) ESBL-producing isolates. A many fold increase in resistance to antibiotics was observed in biofilm. Among ESBL-producers, seven isolates could transfer the $\text{bla}_{\text{CTX-M}}$ gene by conjugation, with transfer frequencies ranging from $2.22 \times 10^{-4}$ to $7.14 \times 10^{-2}$ transconjugants/recipient cell in the planktonic state and from $3.04 \times 10^{-3}$ to $9.15 \times 10^{-1}$ in biofilm. The transfer frequency of $\text{bla}_{\text{CTX-M}}$ was significantly higher in biofilm compared with the planktonic state, and co-transfer of ciprofloxacin resistance was also detected in five isolates. This study demonstrates that biofilm-forming ESBL-producing enteric bacteria with a greater transfer frequency of resistance genes will lead to frequent dissemination of $\beta$-lactam and fluoroquinolone resistance genes in environmental settings. The emergence and spread of such multidrug resistance is a serious threat to animal and public health.

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1. Introduction

In the natural environment, the catalytic and protective conditions offered by life on surfaces facilitate microbial growth and the development of complex communities enclosed within a self-generated extracellular matrix, termed a biofilm. Over the past 20 years, the structural and developmental complexity of biofilms has evoked extensive research to explore its significance in natural and man-made environments. High bacterial density and diversity have been reported in biofilms formed in wastewater [1] and drinking water distribution systems [2].

The ecological success of most enteric bacteria in different environments can be attributed to their preference to aggregate or their ability to form biofilms. Biofilm formation as a survival strategy results in microbes achieving a high degree of stable growth in harsh and fluctuating environmental conditions. Biofilm formation ability by drug-resistant bacteria may result in increased tolerance to toxic substances, including antibiotics, in hospital wastewater where selection pressure is high due to the release of antibiotics into effluents and municipal sewage via patient excreta or direct deposition [3]. Moreover, the strong antibiotic selective pressure leads to an increase in multidrug-resistant (MDR) bacteria both from clinical and environmental sources [4].

The emergence and spread of antibiotic resistance in bacterial pathogens is a global problem to clinicians, drug manufacturing industries and healthcare agencies. Extended-spectrum $\beta$-lactamases (ESBLs) are one of the major determinants of resistance against oxyimino-cephalosporins among Gram-negative bacteria, especially within members of the Enterobacteriaceae. Recently, a report by the World Health Organization (WHO) on antimicrobial
Emergence of ciprofloxacin-resistant extended-spectrum \( \beta \)-lactamase-producing enteric bacteria in hospital wastewater and clinical sources

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**A B S T R A C T**

This study aimed to evaluate the incidence of ciprofloxacin-resistant extended-spectrum \( \beta \)-lactamase (ESBL)-producing enteric bacteria in hospital wastewater and clinical sources. Enteric bacteria, mainly *Escherichia coli*, were isolated from clinical sources (urinary tract and gastrointestinal tract infections; 80 isolates) and hospital wastewater (103 isolates). The antibiotic resistance profile and ESBL production of the isolates were investigated by disc diffusion assay and combined disc diffusion test, respectively. Plasmid profiling was performed by agarose gel electrophoresis, and elimination of resistance markers was performed by a plasmid curing experiment. Antibiotic susceptibility testing revealed a high incidence of \( \beta \)-lactam resistance, being highest to ampicillin (88.0%) followed by amoxicillin, ceftriaxone, cepodoxime, cefotaxime, aztreonam, cefepime and ceftazidime. Among the non-\( \beta \)-lactam antibiotics, the highest resistance was recorded to nalidixic acid (85.7%). Moreover, 50.8% of enteric bacteria showed resistance to ciprofloxacin. Among 183 total enteric bacteria, 150 (82.0%) exhibited multidrug resistance. ESBL production was detected in 78 isolates (42.6%). A significantly higher incidence of ciprofloxacin resistance was observed among ESBL-producing enteric bacteria both in clinical \( (P=0.0015) \) and environmental isolates \( (P=0.012) \), clearly demonstrating a close association between ESBL production and ciprofloxacin resistance. Plasmid profiling of selected ESBL-positive strains indicated the presence of one or more plasmids of varying sizes. Plasmid curing resulted in loss of ciprofloxacin and cephalosporine resistance markers simultaneously from selected ESBL-positive isolates, indicating the close relationship of these markers. This study revealed a common occurrence of ciprofloxacin-resistant ESBL-producing enteric bacteria both in hospital wastewater and clinical sources, indicating a potential public health threat.

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1. Introduction

Hospital wastewater is considered one of the major reservoirs of pathogenic bacteria. Wastewater or natural water supplies into which wastewater has been discharged are likely to contain pathogenic organisms mainly coming from human excreta [1]. Furthermore, antibiotics used in hospitals and their release into effluents and municipal sewage via patient excreta or direct deposition impose a selection pressure on bacteria leading to emergence of resistance to different classes of antibiotics in micro-organisms in the aquatic environment [2]. Besides this, wastewater from hospitals constitutes a route of dissemination of antibiotic-resistant bacteria to human and animal populations through various ecological modes of transmission including food, water and insects vectors. Moreover, in developing countries, the chance of transmission of pathogenic bacteria is more common owing to lack of adequate hygiene, poor water quality and inadequate management of human and hospital wastes [1]. Considering the impact of hospital-acquired infections, national evidence-based guidelines for preventing healthcare-associated infections in National Health Service (NHS) hospitals were commissioned by the Department of Health in England. These guidelines focused on hospital environmental hygiene, hand hygiene, the use of personal protective equipment, and the safe use and disposal of sharps; preventing infections associated with the use of short-term indwelling urethral catheters; and preventing infections associated with central venous catheters [3].
Plant Growth Promoting Activities, Biofilm Formation and Root Colonization by *Bacillus* sp. Isolated from Rhizospheric Soils

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Efficient biofilm forming PGPR is expected to perform better under field conditions due to enhanced root colonization and survival under stress condition. Therefore, in this study 14 isolates of *Bacillus* sp. were selected after random screening based on multiple plant growth promoting traits like phosphate solubilization, production of indole acetic acid, siderophore, HCN, ammonia, EPS and ACC deaminase activity. Biofilm formation was studied *in vitro* by using 96 well polystyrene plate. Based on *in vitro* biofilm formation these isolates were grouped as moderate and strong biofilm former. Two isolates of *Bacillus subtilis* (BD6 and BCH4) identified by 16S rRNA gene sequence analysis exhibited multiple PGP traits but differed in their biofilm forming capacity were evaluated for root colonization and biofilm formation on the root surface. Biofilm was characterized by scanning electron and confocal laser scanning microscopy. Colonization studies revealed that isolate BCH4 colonized wheat roots more strongly compared to BD6 and form a biofilm on the root surface. Inoculation response to wheat showed an increase in plant vegetative growth parameters compared to control in a pot culture assay indicating that BCH4 a strong biofilm former with multiple PGP traits may be considered for further development as bioinoculant.

Key words: Confocal laser scanning microscopy; *Bacillus subtilis*; Biofilms; PGPR; *Triticum aestivum*; 16S rRNA gene analysis; root colonization.

Plant growth promoting rhizobacteria (PGPR) are the diverse class of microbes that inhabit the plant root as ectophytes or endophytes, enhancing the host plant growth by both direct and indirect mechanism. The application of beneficial rhizobacteria can help in reducing dependency on chemical fertilizers in enhancing agriculture production. PGPR can stimulate plant growth by increasing the plant nutrition by several mechanisms like the production of plant growth hormones, nitrogen fixation and phosphate solubilization, siderophore production. Another mechanism of plant growth promotion took place through the biocontrol activity and induced systemic resistance as well as by favoring rhizobia or mycorrhizal symbiosis.

Various species of genus *Bacillus* are well recognized for their plant growth promoting properties. The members of this genus have unique characteristics due to the formation of endospore, production of peptide antibiotics and extracellular enzymes. These properties make this genus survive better in different environments for the long duration, under field condition.

In principle, plant growth promoting agent like the genus *Bacillus* as bioinoculant should build and sustain their critical population size to impart the benefits for efficient plant growth. Therefore, keeping in view of the problem of survival and long term sustenance after inoculation, it is proposed that PGPR with biofilm-forming capability can efficiently colonize, survive and
CHARACTERIZATION OF PAENIBACILLUS DURUS (PNF16) A NEW ISOLATE AND ITS SYNERGISTIC INTERACTION WITH OTHER ISOLATED RHIZOBACTERIA IN PROMOTING GROWTH AND YIELD OF CHICKPEA

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ABSTRACT

Application of PGPR in crop production and protection is well known and can also contribute in reducing use of agrochemicals. However, the performance of PGPR is influenced by various biotic and abiotic factors. Isolating new efficient PGPR strain well adapted to local soil agroclimatic conditions is expected to perform with more consistency. In this study five selected rhizobacteria isolated previously were first tested in vitro for plant growth promoting (PGP) characteristics. One of the isolate PNF16 was identified as Paenibacillus durus by 16S rRNA gene sequence analysis. PNF16 alone and in combination with other rhizobacteria (Azotobacter (AZT), Achromobacter (PNF15), Bacillus (Bc), Pseudomonas (Ps) and Mesorhizobium (IARI)) were tested for plant growth promoting effect under pot conditions in two consecutive years. PNF16 was found to produce 21.7 µg ml⁻¹ of indole acetic acid like substances, hydroxymate type of siderophores (Salicylate type 11 µg ml⁻¹ and benzoate type 6.5 µg ml⁻¹) and solubilized phosphate (405.33 µg ml⁻¹). PNF16 -Mesorhizobium combination was found significantly better compared to other combinations for growth parameters, nodulation and yield of chickpea over control. Similar study was also performed for other tested strain. Significant increase in plant growth (32%), nodulation (43%) compared to untreated control was recorded. Co-inoculation also showed synergy and increased the number of pods per plant, 1000-grain weight, dry matter yield, grain yield and protein content by 23%, 22%, 21%, 18% and 4.4% respectively, compared to control. The results indicated the potential usefulness of PNF16 alone and in combinations in enhancement of nodulation and stimulation of plant growth in chickpea and adapted to soil condition of the region.

Keywords: Paenibacillus; Mesorhizobium; PGPR, plant-microbe interaction, nodulation, chickpea

INTRODUCTION

Interactions between plants and micro-organisms in the rhizosphere can clearly affect crop yields. Rhizobacteria that benefit plant growth and development are called ‘PGPR’. The term ‘PGPR’ was introduced in 1978 by Kloepper and colleagues. Since then a large number of bacteria have been identified and reported as PGPR (Acetobacter, Achromobacter, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Cloridriun, Enterobacter, Flavobacterium, Frankia, Hydrogenophaga, Kluyvera, Microcoleus, Phyllobacterium, Pseudomonas, Rhizobium, Serratia, Staphylococcus, Streptomyces, and Vibrio) (Banash et al., 2005). However commonly used PGPR in field application is limited to only few microorganisms. The plant growth promoting rhizobacteria may enhance plant growth either directly or indirectly. Direct mechanisms include (i) the ability to produce the plant growth regulators (indoleacetic acid, gibberellins, cytokinins and ethylene) (Glick, 2012), (ii) Asymbiotic N₂ fixation (Ahmad et al., 2008), (iii) Solubilization of mineral nutrient like phosphates (Taurian et al., 2010), Indirect mechanisms involve (i) antagonism against phytopathogens (Gururanli et al., 2013), (ii) Production of siderophores (Haas and Defago, 2005), (iii) Production of extra cellular cell wall degrading enzymes for phytopathogens β-1,3 glucanase (Ribeiro and Cardoso, 2012), Chitinase (Ribeiro and Cardoso, 2012), (iv) Antibiotic production (Mazzurier et al., 2009) and (v) cyanide production (Ribeiro and Cardoso, 2012). By modifying the microbial balance in the rhizosphere, PGPR can stimulate plant growth indirectly by inhibiting other deleterious microbes or root pathogens (Berendse et al., 2012). On the other hand, diazotrophs are able to decrease or prevent the deleterious effects of plant pathogens mostly through the synthesis of antibiotic and fungicidal compounds (Mavingui and Heulin 1994; Dobbelare et al., 2003), competition for nutrients (siderophore production) or by the induction of induced systemic resistance (ISR) against pathogens (Timmusk and Wagner 1999; Dobbelare et al., 2003; Gururanli et al., 2013).

A major problem associated with PGPRs is their inability to manifest PGP traits under natural field conditions consistently. This is mainly due to competition with native well adapted strains and specific nutrient limitation (Vasssey, 2003). We hypothesized that selecting a PGPB strains exhibiting multiple traits are expected to most ideal as the probability of expression of one or more PGP traits is higher. It is also expected that indigenous soil bacteria adapted to local soil and agro-climatic conditions exhibiting multiple PGP traits may be more effective under field conditions. We have screened rhizospheric soil in vicinity of Aligarh in northern India (Ahmad et al., 2006). We found a new isolate of Paenibacillus sp. (PNF16) which showed multiple PGP traits and characterized using 16S rRNA gene sequence analysis. The efficacy of PNF16 for plant growth promotion was assayed under pot experiment conditions alone and in combination with other bioinoculant such as Bacillus (Bc), Azotobacter (AZT), Achromobacter (PNF15) and Pseudomonas (Ps) which were previously isolated in our laboratory.

MATERIALS AND METHODS

Isolation and characterization of bacterial isolates

Bacterial isolates PNF16 and other rhizobacteria used in this study were isolated and biochemically characterized using standard methods as described previously (Ahmad et al., 2006; 2008).

Genetic identification of PNF16 by 16S rRNA partial gene sequencing

Single isolated colony PNF16 was inoculated in 5 ml Luria-Bertani (LB) broth and grown at 30 °C for 24 h. Cells were harvested and processed immediately for DNA isolation by standard procedure. The concentration and purity of the DNA preparation were determined by measuring optical density (OD) at 260 nm and ratio at 260/280 nm with a UV-Vis Spectrophotometer. The PCR amplification of almost full-length 16S rRNA gene was carried out with eubacterial specific
Degradation of Reactive Black 5 dye by a newly isolated bacterium *Pseudomonas* *entomophila* BS1

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Molecular and insecticidal characterization of Vip3A protein producing *Bacillus thuringiensis* strains toxic against *Helioverpa armigera* (Lepidoptera: Noctuidae)

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Protective effect of *Lepidium sativum* seed extract against hydrogen peroxide-induced cytotoxicity and oxidative stress in human liver cells (HepG2)

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Abstract

**Context:** Garden cress (*Lepidium sativum* (Brassicaceae)) has been widely used to treat a number of ailments in traditional medicine. The pharmacological and preventive potential of *Lepidium sativum*, such as anti-inflammatory, antipyretic, antihypertensive, anti-asthmatic, anticancer, and anti-oxidant, are well known.

**Objective:** The present investigation was designed to study the protective effects of chloroform extract of *Lepidium sativum* seed (LSE) against oxidative stress and cytotoxicity induced by hydrogen peroxide (H₂O₂) in human liver cells (HepG2).

**Materials and methods:** Cytotoxicity of LSE and H₂O₂ was identified by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), neutral red uptake (NRU) assays, and morphological changes in HepG2. The cells were pre-exposed to biologically safe concentrations (5–25 mg/ml) of LSE for 24 h, and then cytotoxic (0.25 mM) concentration of H₂O₂ was added. After 24 h of the exposures, cell viability by MTT, NRU assays, and morphological changes in HepG2 were evaluated. Further, protective effects of LSE on reactive oxygen species (ROS) generation, mitochondrial membrane potential (MMP), lipid peroxidation (LPO), and reduced glutathione (GSH) levels induced by H₂O₂ were studied.

**Results:** Pre-exposure of LSE significantly attenuated the loss of cell viability up to 48% at 25 mg/ml concentration against H₂O₂ (LD₅₀ value = 2.5 mM). Results also showed that LSE at 25 mg/ml concentration significantly inhibited the induction of ROS generation (45%) and LPO (56%), and increases the MMP (55%) and GSH levels (46%).

**Discussion and conclusion:** The study suggests the cytoprotective effects of LSE against H₂O₂-induced toxicity in HepG2. The results also demonstrate the anti-oxidative nature of LSE.

Keywords

Glutathione, lipid peroxidation, MMP, morphological changes, ROS generation

Introduction

Oxidative stress plays a significant role in the etiology of variety of human diseases (Dhalla et al., 2000; Rahman, 2005). The role of oxidative stress in liver cells induced by various toxins is also well known (Rodeiro et al., 2008). Reports showed that overproduction of reactive oxygen species (ROS) plays a major role in the hepatocarcinoma (Lima et al., 2006; Zhang et al., 2011), which leads to cellular damage (Lin et al., 2007; Zhang et al., 2012). A number of *in vitro* studies have demonstrated that oxidative stress induced by chemical oxidants, such as hydrogen peroxide (H₂O₂), leads to cell death (Cai et al., 2008; Hwang & Yen, 2008; Kim et al., 2009; Siddiqui et al., 2011). H₂O₂ has also been reported to induce apoptotic changes, which subsequently lead to death in a variety of cell systems (Jung et al., 2006; Kanno et al., 2003; Sattayasai et al., 2013), including human liver cells (HepG2) (Alia et al., 2005; Chen et al., 2011). Therefore, we have selected H₂O₂ to induce the oxidative stress-mediated cytotoxicity in the HepG2.

Garden cress (*Lepidium sativum* (Brassicaceae)) has been widely used to treat a number of ailments in traditional system of medicine. *Lepidium sativum* is a fast-growing edible herb belonging to the family Brassicaceae (Cruciferae) or mustard family, and is being cultivated as culinary vegetable in North America, Europe, and all over Asia including India (Al-Sheddi et al., 2013; Nadkarni, 1976). The pharmacological and preventive properties of *L. sativum*, such as antioxidant (Yadav et al., 2010), anti-inflammatory, anticoagulant (Al-Yahya et al., 1994), antidiabetic (Eddoaks et al., 2005), antiarrrheal (Manohar et al., 2009), antihypertensive, diuretic (Mohamed et al., 2003; Umang et al., 2009), antiirheumatic (Ahsan et al., 1989), anti-asthmatic (Paranjape & Mehta, 2006), chemoprotective (Fekadu et al, 2007).
Role of plant growth promoting rhizobacteria in sustainable production of vegetables: Current perspective

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A B S T R A C T

In order to optimize the crop production and hence, to achieve food security, synthetic fertilizers have largely been used in high input agronomic practices to offset major and sometimes minor nutrient deficiencies of soils with concomitant intensification in food production. When used repeatedly in horticultural practices, such environmentally unfriendly fertilizers have deleteriously impacted soil fertility and consequently, the crop productivity. Taking these threats into account, scientists are desperate to find inexpensive, environmentally benign and easy to operate options to overcome fertilizer toxicity problems. In this regard, plant growth promoting rhizobacteria (PGPR) have magnetize the agrarian communities due in part to their low cost, easy access and simple mode of application. Broadly, PGPR when used either alone or in consortia, have resulted in tremendous positive impact on horticultural production. Among horticultural crops, the interest in quality of vegetables in recent times among consumers has increased worldwide. The results of studies conducted so far worldwide on the impact of PGPR carrying numerous multi-functional plant growth promoting activities on horticultural crops especially vegetables grown distinctively in different production systems is discussed and considered. The review will conclude by identifying several PGPR for future researches aiming to improve the health and quality of vegetables grown in different production systems. Also, the findings presented here are likely to reduce the use of chemical fertilizers in horticultural practices and to protect human health (via food chain) from the ill effect of fertilizers used in different agronomic environment.

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Contents

1. Plant growth promoting rhizobacteria (PGPR)—definition, origin, introduction ............................................... 232
2. PGPR inoculant development and production ........................................................................................................ 232
3. PGPR improve vegetable production ...................................................................................................................... 233
   3.1. A general perspective ................................................................................................................................... 233
   3.2. Sustainable production .................................................................................................................................. 233
       3.2.1. Free living PGPR .................................................................................................................................... 233
       3.2.2. Rhizobia .............................................................................................................................................. 234
4. Examples of PGPR effects on important vegetable crops ...................................................................................... 234
   4.1. Potato (Solanum tuberosum) ....................................................................................................................... 234
   4.2 Tomato (Solanum lycopersicum) ................................................................................................................... 235
   4.3 Eggplant (Solanum melongena) .................................................................................................................... 235
   3.6.4 Pepper (Capsicum annuum) .................................................................................................................... 236
   4.5 Cucumber (Cucumis sativus) ......................................................................................................................... 236
   4.6 Cabbage (Brassica oleracea) ....................................................................................................................... 236
   4.7 Radish and daikon (Raphanus sativus) .......................................................................................................... 236

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In vitro assay of molecules and anti-phytopathogenic activity of free living PGPR

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Microwave Accelerated Green Synthesis of Stable Silver Nanoparticles with Eucalyptus globulus Leaf Extract and Their Antibacterial and Antibiofilm Activity on Clinical Isolates

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Abstract

A simple and rapid microwave assisted method of green synthesis of silver nanoparticles (AgNPs) was developed using aqueous leaf extract of Eucalyptus globulus (ELE), and their antibacterial and antibiofilm potential investigated. With this aim, the aqueous solutions of ELE and AgNO₃ (1 mM) were mixed (1:4 v/v), and microwave irradiated at 2450 Mhz, for 30 sec. The instant color change of the ELE-AgNO₃ mixture from pale yellow to dark brown indicated ELE-AgNPs synthesis. The intensity of peak at 428 nm in UV-Vis spectra, due to the surface plasmon resonance of AgNPs, varied with the amount of ELE, AgNO₃ concentration, pH and time of incubation. The biosynthesized ELE-AgNPs were characterized by UV-visible spectroscopy, XRD, TEM, SEM-EDX, FTIR and TGA analyses. The size of ELE-AgNPs was determined to be in range of 1.9–4.3 nm and 5–25 nm, with and without microwave treatment, respectively. SEM exhibited the capping of AgNPs with the ELE constituents, and validated by FTIR analysis. The FTIR data revealed the presence of plant organic constituents and metabolites bound to ELE-AgNPs, which contributes for their stability. The antimicrobial activity of ELE-AgNPs was assessed by growth and biofilm inhibition of extended spectrum β-lactamase (ESBL) producing Pseudomonas aeruginosa, Escherichia coli and methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive Staphylococcus aureus (MSSA) clinical bacterial isolates. The results demonstrated that S. aureus were more sensitive to ELE-AgNPs than E. coli and P. aeruginosa. MRSA exhibited higher sensitive than MSSA, whereas P. aeruginosa were more sensitive than E. coli to ELE-AgNPs treatment. Also, significant (83 ± 3% and 84 ± 5%) biofilm inhibition was observed in case of S. aureus and P. aeruginosa, respectively. The results elucidated environmentally friendly, economical and quick method for production of colloidal bio-functionalized ELE-AgNPs, for effectual clinical applications, as broad spectrum antibacterial agents and biofilm inhibitors.
Rhamnolipids functionalized AgNPs-induced oxidative stress and modulation of toxicity pathway genes in cultured MCF-7 cells

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A B S T R A C T
Rhamnolipids extracted from Pseudomonas aeruginosa strain JS-11 were utilized for synthesis of stable silver nanoparticles (Rh-AgNPs). The Rh-AgNPs (23 nm) were characterized by Fourier transform infrared (FTIR) spectroscopy, atomic force microscopy (AFM) and transmission electron microscopy (TEM). The cytotoxicity assays suggested significant decrease in viability of Rh-AgNPs treated human breast adenocarcinoma (MCF-7) cells, compared with normal human peripheral blood mononuclear (PBMM) cells. Flow cytometry data revealed 1.25-fold (p < 0.05) increase in the fluorescence of 2,7′-dichlorofluorescin (DCF) at 0.25 μg/mL. However, at Rh-AgNPs concentrations of 0.5 and 1.0 μg/mL, much lesser fluorescence was noticed, which is attributed to cell death. Results with the fluorescent probe Rh123 demonstrated change in inner mitochondrial membrane and dissipation of membrane potential. The cell cycle analysis suggested 19.9% (p < 0.05) increase in sub-G1 peak with concomitant reduction in G1 phase at 1 μg/mL of Rh-AgNPs, compared to 2.7% in untreated control. The real-time RT2 Profiler™ PCR array data elucidated the overexpression of seven oxidative stress and DNA damage pathways genes viz. BAX, BCL2, Cyclin D1, DNAJA1, E2F transcription factor 1, GPIX1 and HSPA4, associated with apoptosis signaling, proliferation and carcinogenesis, pro-inflammatory and heat shock responses in Rh-AgNPs treated cells. Thus, the increased ROS production, mitochondrial damage and appearance of sub-G1 (apoptotic) population suggested the anti-proliferative activity, and role of oxidative stress pathway genes in Rh-AgNPs induced death of MCF-7 cancer cells.

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1. Introduction

Development of a reliable, eco-friendly and toxicity-free synthesis of metal nanoparticles (NPs) is an important aspect of nanotechnology research [1]. Lately, the biomimetic and green synthesis of AgNPs using polymer matrices such as starch [2], chitosan [3], cyclodextrins [4], and microbial biomass [5–7] has been extensively pursued. Micronal synthesis of NPs yields stable particles due to protein capping and interaction with other reducing agents such as nitrate reductase [8], naphthoquinones [9], and anthraquinones [10], secreted by the organisms. Furthermore, synthetic chemicals such as amine and carboxylate surfactants [11], cationic cetylpyridinium, or anionic sodium dodecyl sulphate, or non-ionic Brij 56 [12] have also been used for NPs synthesis. These surfactants are tension-active molecules, amphipathic in nature with both hydrophilic and hydrophobic moieties, and exhibit surface-active properties. With the increasing demand for greener bioprocesses and novel enhancers for NPs synthesis, the biosurfactants, and/or biosurfactant producing microbes are emerging as an alternate source. Thus, biosurfactants with the high surface activity and low critical micelle concentrations (CMC) are regarded as promising substitutes for synthetic surfactants [13]. Several microorganisms like bacteria, fungi, yeasts, and algae are good sources of biosurfactants and offer many advantages over their chemical counterparts. Therefore, the biosurfactant mediated synthesis of NPs is regarded as a clean, non-toxic, and environmentally acceptable “green chemistry” procedure, resulting in reduced NPs aggregation and uniform morphology. Furthermore, the lower toxicity, higher biodegradability, better environmental compatibility, higher foaming, high selectivity and specific activity at extreme temperatures, pH, and salinity [14] are some added advantages over the chemical surfactants.

In this context, the natural rhamnolipids, a subclass of glycolipids produced by bacteria, could serve as simple and economical

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ZnO and TiO$_2$ nanoparticles as novel antimicrobial agents for oral hygiene: a review

Shams Tabrez Khan · Abdulaziz A. Al-Khedhairy · Javed Musarrat

Abstract Oral cavity is inhabited by more than 25,000 different bacterial phylotypes; some of them cause systemic infections in addition to dental and periodontal diseases. Emergence of multiple antibiotic resistance among these bacteria necessitates the development of alternative antimicrobial agents that are safe, stable, and relatively economic. This review focuses on the significance of metal oxide nanoparticles, especially zinc oxide and titanium dioxide nanoparticles as supplementary antimicrobials for controlling oral infections and biofilm formation. Indeed, the ZnO NPs and TiO$_2$ NPs have exhibited significant antimicrobial activity against oral bacteria at concentrations which is not toxic in in vivo toxicity assays. These nanoparticles are being produced at an industrial scale for use in a variety of commercial products including food products. Thus, the application of ZnO and TiO$_2$ NPs as nanoantibiotics for the development of mouthwashes, dental pastes, and other oral hygiene materials is envisaged. It is also suggested that these NPs could serve as healthier, innocuous, and effective alternative for controlling both the dental biofilms and oral planktonic bacteria with lesser side effects and antibiotic resistance.

Keywords ZnO and TiO$_2$ nanoparticles · Oral bacteria · Biofilm · Nanoantibiotics · Antibiotic resistance · Nanomedicine · Health effects

Introduction

The oral microbiome is a complex ecosystem consisting of about 25,000 different bacterial phylotypes as revealed by deep sequencing of human oral microbiome, traditional cultivation, and cloning approaches (Jenkinson et al. 2011; Keijser et al. 2008; Liu et al. 2012; Paster et al. 2006). The proliferation of pathogenic bacteria within the mouth gives rise to periodontitis, an inflammatory disease, which also constitutes a risk factor for other systemic diseases (Zbinden et al. 2012) such as endocarditis and colorectal cancer (Han and Wang 2013). Therefore, one of the most urgent and important biomedical challenges of our times is to clarify the role of microbial communities in human health (Belda-Ferre et al. 2011; Turnbaugh et al. 2007; Ximénez-Fyvie et al. 2000). Unfortunately, the antibiotic therapies have rendered these bacteria resistant to traditional antibiotics (Leistevuo et al. 2000; Sweeney et al.
Anticancer Activity of Chloroform Extract and Sub-fractions of *Nepeta deflersiana* on Human Breast and Lung Cancer Cells: An In vitro Cytotoxicity Assessment

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**ABSTRACT**

**Background:** Cancer is one of the major causes of death worldwide. The plant-derived natural products have received considerable attention in recent years due to their diverse pharmacological properties including anticancer effects. *Nepeta deflersiana* (ND) is used in the folk medicine as antiseptic, carminative, antimicrobial, antioxidant, and for treating rheumatic disorders. However, the anticancer activity of ND chloroform extract has not been explored so far. **Objectives:** The present study was aimed to investigate the anticancer activities of chloroform *Nepeta deflersiana* extract and various sub-fractions (ND-1–ND-15) of ND against human breast cancer cells (MCF-7) and human lung cancer cells (A-549). **Materials and Methods:** The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide and neutral red uptake assays, and cellular morphological alterations using phase contrast light microscope were studied. Cells were exposed with 10–1000 µg/ml of sub-fractions of ND for 24 h. **Results:** Results showed that selected sub-fractions of the chloroform extract significantly reduced the cell viability of MCF-7 and A-549 cells, and altered the cellular morphology in a concentration-dependent manner. Among the sub-fractions, ND-10 fraction showed relatively higher cytotoxicity compared to other fractions whereas, ND-1 did not cause any cytotoxicity even at higher concentrations. The A-549 cells were found to be more sensitive to growth inhibition by all the extracts as compared to the MCF-7 cells. **Conclusion:** The present study provides preliminary screening of anticancer activities of chloroform extract sub-fractions of ND, which can be further used for the development of a potential therapeutic anticancer agent. **Key words:** A-549 cells, anticancer, cytotoxicity, MCF-7 cells, *Nepeta deflersiana*

**SUMMARY**

- Nepeta deflersiana extract exhibit cytotoxicity and altered the cellular morphology. Sub-fractions of the chloroform extract of *Nepeta deflersiana* reduced the cell viability of MCF-7 and A-549 cells. Among the sub-fractions, ND-10 fraction showed relatively higher cytotoxicity. The A-549 cells were found to be more sensitive as compared to the MCF-7 cells.

**INTRODUCTION**

Cancer is a life-threatening disease, accounted for 8.2 million deaths, in 2012.[1,2] According to World Health Organization, the annual cancer cases will rise from 14 million in 2012 to 22 million within next two decades.[2] It is second most occurring disease after cardiovascular disease and causes a great burden to both single human lives and the society as a whole.[3] Although there has been good progress in the development of prevention and treatment of cancer, the successful treatment of cancer still remains a challenge. Cancer cells often adapt to develop resistance to commonly used chemotherapeutic agents. Therefore, it is important to develop novel chemotherapeutic agents, which are more potent tumor-selective cytotoxic agents and can circumvent drug-resistant cancer cells. Natural products have for long played an important role in drug discovery, especially in the area of cancer pharmacology. Many natural or natural based anti-tumor drugs such as bleomycin, doxorubicin, mitomycin, vinblastine, vincristine, etoposide (VP16), topotecan, irinotecan, paclitaxel, and combretastatin have been clinically used in recent years.[4] *Nepeta* (family: Lamiaceae) is a large genus that is composed of about 250–300 annual and perennial species.[5] In the Kingdom of Saudi Arabia, there are two species of the genus *Nepeta*. *Nepeta deflersiana* (ND) is found in Saudi Arabia and Yemen, whereas *Nepeta sheliea* is found only in the northern Hijaz mountains of Saudi Arabia.[6]

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Molybdenum nanoparticles-induced cytotoxicity, oxidative stress, G2/M arrest, and DNA damage in mouse skin fibroblast cells (L929)

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**Abstract**

The present investigation was aimed to study the cytotoxicity, oxidative stress, and genotoxicity induced by molybdenum nanoparticles (Mo-NPs) in mouse skin fibroblast cells (L929). Cells were exposed to different concentrations (1–100 μg/ml) of Mo-NPs (size 40 nm) for 24 and 48 h. After the exposure, different cytotoxicity assays (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT; neutral red uptake, NRU; and cellular morphology) and oxidative stress markers (lipid peroxidation, LPO; glutathione, GSH; and catalase) were studied. Further, Mo-NPs-induced intracellular reactive oxygen species (ROS) generation, mitochondrial membrane potential (MMP), cell cycle arrest, and DNA damage were also studied. L929 cells treated with Mo-NPs showed a concentration- and time-dependent decrease in cell viability and a loss of the normal cell morphology. The percentage cell viability was recorded as 25%, 42%, and 58% by MTT assay and 24%, 46%, and 56% by NRU assay at 25, 50, and 100 μg/ml of Mo-NPs, respectively after 48 h exposure. Furthermore, the cells showed a significant induction of oxidative stress. This was confirmed by the increase in LPO and ROS generation, as well as the decrease in the GSH and catalase levels. The decrease in MMP also confirms the impaired mitochondrial membrane. The cell cycle analysis and comet assay data revealed that Mo-NPs induced G2/M arrest and DNA damage in a concentration-dependent manner. Our results demonstrated, for the first time, Mo-NPs induced cytotoxicity, oxidative stress and genotoxicity in L929 cells. Thus, data suggest the potential hazardous nature of Mo-NPs.

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1. Introduction

Over the past few years, there is a rapid development in the field of nanotechnology worldwide due to their wide applications in the industry and biomedicine [1]. Nanomaterials possessing novel physical and chemical functional properties have been used in the manufacture of unique devices [2,3]. The major toxicological concerns of these manufactured nanoparticles (NPs) are their distinct properties, such as small size, high number per given mass, large specific surface area, generation of free radicals [4], redox active nature [2], and transporting into mitochondria through the cell membranes [5]. Although, most of the NPs have been shown to induce potential toxicity [6], there is a serious lack of information concerning the impact of these NPs on the environment and the human health. It is well established that NPs can affect different cellular activities [7–9] and can cause toxicity in various organs [10–12]. Numerous studies showed that NPs can cause cytotoxicity, genotoxicity and apoptotic cell death [13–15] through lysosomal membrane destabilization and lipid peroxidation [16]. Although little is known about the mechanism(s) of nanoparticles toxicity, free oxygen radical generation is often used to explain toxicity associated with NPs exposure [17]. Free oxygen radical elicits a wide variety of physiological and cellular events including cellular stress, inflammation, DNA damage, and apoptosis [18]. Many studies have also reported that NPs have the potential to induce...
CoO Thin Nanosheets Exhibit Higher Antimicrobial Activity Against Tested Gram-positive Bacteria Than Gram-negative Bacteria

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Abstract – Envisaging the role of Co in theranautics and biomedicine it is immensely important to evaluate its antimicrobial activity. Hence in this study CoO thin nanosheets (CoO-TNs) were synthesized using wet chemical solution method at a very low refluxing temperature (90 °C) and short time (60 min). Scanning electron microscopy of the grown structure revealed microflowers (2–3 µm) composed of thin sheets petals (60–80 nm). The thickness of each individual grown sheet varies from 10–20 nm. Antimicrobial activities of CoO-TNs against two Gram positive bacteria (Micrococcus luteus, and Staphylococcus aureus), and two Gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa) were determined. A 98% and 65% growth inhibition of M. luteus and S. aureus respectively, was observed with 500 µg/ml of CoO-TNs compared to 39 and 34% growth inhibition of E. coli and P. aeruginosa, respectively with the same concentration of CoO-TNs. Hence, synthesized CoO-TNs exhibited antimicrobial activity against Gram negative bacteria and an invariably higher activity against tested Gram positive bacteria. Therefore, synthesized CoO-TNs are less prone to microbial infections.

Key words: Nanostructures, CoO-TNs, Antibacterial Activity, Theranautics

1. Introduction

Metallic nanoparticles (NPs) with different structures and properties represent an enormous opportunity in medical field due to their small size and shape [1-3]. Therefore, many new NPs with interesting properties and potential applications in theranautics and biomedicine are being synthesized [4-6]. Among various nanocompounds, cobalt nanoparticles (CoNPs) are of special interest as they display a wealth of size-dependent catalytic, electronic, magnetic, and structural properties. Owing to these properties, CoO-NPs offer powerful tools for research activity and have been proposed for a number of applications in different fields including theranautics and biomedicine as reviewed by Akbarzadeh et al. [7]. Wang et al., [8] designed a novel glucose biosensor using nanoscaled cobalt phthalocyanine (NanCoPe)-glucose oxidase (GOD) biocomposite. Recently Kainz et al., synthesized carbon coated cobalt nanoparticles for their potential use in biomedicine [9]. Co and CoPt are traditionally synthesized using carbonyl pyrolysis [10-12]. However, other methods such as solution phase metal salt reduction are also being used for the synthesis of CoO nanocrystals. CoO nanocrystals are being studied for hydrogel-olysis [13], CO oxidation [14], and for their electrochemical behaviors [15]. Wang et al. reported the synthesized octahedral CoO nanocrystals of 200 nm or even larger and studied their electrochemical performance [16]. Zhang et al. has also reported synthesis of CoO nano crystals with various morphologies [17]. As it is difficult to obtain pure CoO, techniques like laser vaporization controlled condensation are being used for the synthesis of CoO nano particles [18].

The present work, reports new simple and mild procedure for the synthesis of pure CoO microflowers composed with thin sheets (CoO-TNs), using cobalt nitrate hexahydrate (Co(NO₃)₂·6H₂O) and sodium hydroxide (NaOH). Considering, the proposed use of cobalt based NPs in biomedicine and theranautics, it is important to evaluate their antimicrobial activities as many biomaterials are prone to microbial infections [19-20] posing a serious health risk. To the best of our knowledge, there is only one report on the antimicrobial activity of CoO nanostructures [21]. This study focuses on the modulation of synthetic parameters and arrangement of cobalt microflowers composed with thin nanosheets and their antimicrobial activity.

2. Experimental

2.1. Material and methods

2.1.1. Synthesis of cobalt oxide micro-flowers composed with thin nanosheets (CoO-TNs)

CoO-TNs were synthesized using cobalt nitrate hexahydrate (Co(NO₃)₂).
Comparative cytotoxicity of dolomite nanoparticles in human larynx HEp2 and liver HepG2 cells

Maqsood Ahameda*, Hisham A. Alhadlaqb, Javed Ahmadc, Maqsood A. Siddiquic, Shams T. Khan, Javed Musarratd and Abdulaziz A. Al-Khedhairyc

ABSTRACT: Dolomite is a natural mineral of great industrial and commercial importance. With the advent of nanotechnology, natural minerals including dolomite in the form of nanoparticles (NPs) are being utilized in various applications to improve the quality of products. However, safety or toxicity information of dolomite NPs is largely lacking. This study evaluated the cytotoxicity of dolomite NPs in two widely used in vitro cell culture models: human airway epithelial (HEp2) and human liver (HepG2) cells. Concentration-dependent decreased cell viability and damaged cell membrane integrity revealed the cytotoxicity of dolomite NPs. We further observed that dolomite NPs induce oxidative stress in a concentration-dependent manner, as indicated by depletion of glutathione and induction of reactive oxygen species (ROS) and lipid peroxidation. Quantitative real-time PCR data demonstrated that the mRNA level of tumor suppressor gene p53 and apoptotic genes (bax, CASP3 and CASP9) were up-regulated whereas the anti-apoptotic gene bcl-2 was down-regulated in HEp2 and HepG2 cells exposed to dolomite NPs. Moreover, the activity of apoptotic enzymes (caspase-3 and caspase-9) was also higher in both kinds of cells treated with dolomite NPs. It is also worth mentioning that HEp2 cells seem to be marginally more susceptible to dolomite NPs exposure than HepG2 cells. Cytotoxicity induced by dolomite NPs was efficiently prevented by N-acetyl cysteine treatment, which suggests that oxidative stress is primarily responsible for the cytotoxicity of dolomite NPs in both HEp2 and HepG2 cells. Toxicity mechanisms of dolomite NPs warrant further investigations at the in vivo level. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: dolomite nanoparticles; human health; cytotoxicity; oxidative stress; apoptosis

Introduction

Dolomite is a natural mineral composed of calcium magnesium carbonate [CaMg(CO3)2]. Usually minerals are named after a geographic locality where they occur, however, dolomite was named after French geologist Deodat de Dolomieu (1750–1801) (Janez, 2001). Dolomite is an important raw material used in various applications including road and building construction materials, glass manufacturing, metallurgy, and ceramic glazes (British Geological Survey) on Dolomites, 2006; Roberts, 1981). Dolomite is relatively soft and easily crushed to a fine powder, which is used as agricultural lime by farmers to reduce soil acidity and also to adjust magnesium deficiencies (Chen et al., 2006). Dolomite is also used for a range of filler applications in plastics, paints, rubbers and adhesives (BGS (British Geological Survey) on Dolomites, 2006). Dolomite is even utilized in cosmetics such as facial creams, baby powder and toothpaste (Slomski and Odle, 2005). More importantly, dolomite is being used for its potential ability to act as a calcium and magnesium supplement (Mizoguchi et al., 2005), although its safety and effectiveness as such has yet to proven. With the advent of nanotechnology, many natural minerals in the form of nanoparticles (NPs, 1–100 nm) are being utilized in various industrial and commercial applications to improve the quality of products (Akhtar et al., 2014; Berlo et al., 2009; Buseck and Pösfai, 1999; Hochella et al., 2008). Therefore, an improved understanding of the potential risks, comprising of exposure and hazard assessments, associated with exposure to such NPs is necessary to check its toxicity or safety (Kim et al., 2011; Maynard et al., 2006).

The materials at nano-scale shows new and different properties compared with what they exhibit on a macro-scale, enabling unique applications (Jos et al., 2009; Oberdorster et al., 2005). The unique physicochemical properties of NPs come from their high surface-area-to-volume ratio. They also have a considerably higher percentage of atoms on their surface compared with bulk particles, which can make them more reactive. These

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Comparison on the molecular response profiles between nano zinc oxide (ZnO) particles and free zinc ion using a genome-wide toxicogenomics approach

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Abstract Increasing production and applications of nano zinc oxide particles (nano-ZnO) enhances the probability of its exposure in occupational and environmental settings, but toxicity studies are still limited. Taking the free Zn ion (Zn²⁺) as a control, cytotoxicity of a commercially available nano-ZnO was assessed with a 6-h exposure in Escherichia coli (E. coli). The fitted dose-cytotoxicity curve for ZnCl₂ was significantly sharper than that from nano-ZnO. Then, a genome-wide gene expression profile following exposure to nano-ZnO was conducted by use of a live cell reporter assay system with library of 1820 modified green fluorescent protein (GFP)-expressing promoter reporter vectors constructed from E. coli K12 strains, which resulted in 387 significantly altered genes in bacterial (p<0.001). These altered genes were enriched into ten biological processing and two cell components (p<0.05) terms through statistical hypergeometric testing, strongly suggesting that exposure to nano-ZnO would result a great disturbance on the functional gene product synthesis processing, such as translation, gene expression, RNA modification, and structural constituent of ribosome. The pattern of expression of 37 genes altered by nano-ZnO (fold change>2) was different from the profile following exposure to 6 mg/L of free zinc ion. The result indicates that these two Zn forms might cause toxicity to bacterial in different modes of action. Our results underscore the importance of understanding the adverse effects elicited by nano-ZnO after entering aquatic environment.

Keywords Nanoparticle · Cytotoxicity · Gene expression · Gene set enrichment analysis · Pathways

Introduction Nano zinc oxide particles (nano-ZnO) are widely used in many different industrial and consumer products including sunscreen products (Schilling et al. 2010), feed industry (Sunder et al. 2007), rubber (Vladuta et al. 2010), and antibacterial agents (Brayner et al. 2006). Increased production and application of nano-ZnO enhances the probability of its exposure in occupational and environmental settings. Several studies have demonstrated toxicity of nano-ZnO in a wide array of organisms including bacteria, algae, yeast, protozoa, and zebrafish (Franklin et al. 2007; Kasemets et al. 2009; Mortimer et al. 2010; Zhu et al. 2008). However, there are contradictory reports, where the toxicity has been attributed to the potential dissolvability of nano-ZnO into free zinc ions (Zn²⁺) (Brunner et al. 2006; Deng et al. 2009; Nel et al. 2006), while others suggested that dissolution of particles into Zn²⁺ is
Article

Novel All Trans-Retinoic Acid Derivatives: Cytotoxicity, Inhibition of Cell Cycle Progression and Induction of Apoptosis in Human Cancer Cell Lines

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Abstract: Owing to the pharmacological potential of ATRA (all trans-retinoic acid), a series of retinamides and a 1-(retinoyl)-1,3-dicyclohexylurea compound were prepared by reacting ATRA with long chain alkyl or alkenyl fatty amines by using a 4-demethylaminopyridine (DMAP)-catalyzed N,N′-dicyclohexylcarbodiimide (DCC) coupling. The successful synthesis of the target compounds was demonstrated using a range of spectroscopic techniques. The cytotoxicity of the compounds was measured along with their ability to induce cell cycle arrest and apoptosis in human cancer cell lines MCF-7 (breast cancer) and HepG2 (liver cancer) and normal human cell line HEK293 (embryonic kidney). The results of cytotoxicity and flow cytometry data showed that the compounds had a moderate to strong effect against MCF-7 and HepG2 cells and were less toxic to HEK293 cells. N-oleyl-retinamide was found to be the most potent anticancer agent and was more effective against MCF-7 cells than HepG2 cells.
RESEARCH ARTICLE

Portulaca oleracea Seed Oil Exerts Cytotoxic Effects on Human Liver Cancer (HepG2) and Human Lung Cancer (A-549) Cell Lines

Ebtesam Saad Al-Sheddi¹, Nida Nayyar Farshori¹, Mai Mohammad Al-Oqail¹, Javed Musarrat²³, Abdulaziz Ali Al-Khedhairy²³, Maqsood Ahmed Siddiqui²³*¹

Abstract

Portulaca oleracea (Family: Portulacaceae), is a well known for its anti-inflammatory, antioxidative, antibacterial, and anti-tumor activities. However, cytotoxic effects of seed oil of Portulaca oleracea against human liver cancer (HepG2) and human lung cancer (A-549) cell lines have not been studied previously. Therefore, the present study was designed to investigate the cytotoxic effects of Portulaca oleracea seed oil on HepG2 and A-549 cell lines. Both cell lines were exposed to various concentrations of Portulaca oleracea seed oil for 24h. After the exposure, percentage cell viability was studied by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT), neutral red uptake (NRU) assays, and cellular morphology by phase contrast inverted microscopy. The results showed a concentration-dependent significant reduction in the percentage cell viability and an alteration in the cellular morphology of HepG2 and A-549 cells. The percentage cell viability was recorded as 73%, 63%, and 54% by MTT assay and 76%, 61%, and 50% by NRU assay at 250, 500, and 1000 μg/ml, respectively in HepG2 cells. Percentage cell viability was recorded as 82%, 72%, and 64% by MTT assay and 83%, 68%, and 56% by NRU assay at 250, 500, and 1000 μg/ml, respectively in A-549 cells. The 100 μg/ml and lower concentrations were found to be non cytotoxic to A-549 cells, whereas decrease of 14% and 12% were recorded by MTT and NRU assay, respectively in HepG2 cells. Both HepG2 and A-549 cell lines exposed to 250, 500, and 1000 μg/ml of Portulaca oleracea seed oil lost their normal morphology, cell adhesion capacity, become rounded, and appeared smaller in size. The data from this study showed that exposure to seed oil of Portulaca oleracea resulted in significant cytotoxicity and inhibition of growth of the human liver cancer (HepG2) and human lung cancer (A-549) cell lines.

Keywords: Portulaca oleracea - cytotoxicity - cellular morphology - cell viability - cancer cells

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Introduction

Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012 (IARC, 2012). Lung cancer (1.59 million deaths) and liver cancer (745 000 deaths) are the most common causes of cancer death (Fazeli et al., 2012; IARC, 2012). An accurate diagnosis of cancer diseases is much essential for adequate and effective treatment because of the specific type of cancer. Every cancer type requires a specific course of therapy which includes one or more modalities such as surgery, and/or radiotherapy, and/or chemotherapy. Chemotherapy is a category of cancer treatment that uses chemical substances, especially one or more anti-cancer drugs (chemotherapeutic agents). It is now considered as the most effective method of cancer treatment. Among the alternative traditional approaches, various plant products classified as alkaloids, saponins, triterpenes, glycosides, and polyphenols have shown very promising anticancer properties in both in vitro and in vivo (Huang and Zou, 2011; Kma, 2013).

Portulaca oleracea (Family: Portulacaceae), is an annual green herbaceous medicinal plant widespread in temperate and tropical regions of the world (Yang et al., 2009). Portulaca oleracea is a fascinating plant recognised in most cultures for its extensive nutritional benefits. It has been used traditionally as a vegetable for human consumption (Bidhendi et al., 2014). The pharmacological potential of the Portulaca oleracea, such as anti-inflammatory (Chan et al., 2000), antioxidative (Dkhil et al., 2011), anti-bacterial (Zhang et al., 2002), skeletal muscle relaxant (Parry et al., 1993), wound-healing (Rashed et al., 2003), and in vitro anti-tumor (Yoon et al., 1999) activities have been reported. Recently we have also reported that seed extract of Portulaca oleracea induced cytotoxicity against human liver cancer cells (Farshori et al., 2014). Thus, the present investigation was carried out to study the anticancer activity of Portulaca

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Cytotoxic Effects of Portulaca oleracea Seed Oil Against Cancer Cell Lines

Green synthesis of Al₂O₃ nanoparticles and their bactericidal potential against clinical isolates of multi-drug resistant Pseudomonas aeruginosa

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Abstract The high prevalence of extended-spectrum β-lactamases (76.3 %) and metallo-β-lactamases (7.3 %) amongst the bacteria Pseudomonas aeruginosa is a critical problem that has set forth an enormous therapeutic challenge. The suggested role of nanoparticles as next generation antibiotics, and inadequate information on antibacterial activity of aluminium oxide nanoparticles has led us to investigate the green synthesis of aluminium oxide nanoparticles (Al₂O₃ NPs) using leaf extracts of lemongrass and its antibacterial activity against extended-spectrum β-lactamases and metallo-β-lactamases clinical isolates of P. aeruginosa. The synthesized Al₂O₃-NPs were characterized by scanning electron microscopy, high resolution-transmission electron microscopy, atomic force microscopy, X-ray diffraction, Zeta potential, and differential light scattering techniques. The X-ray diffraction data revealed the average size of the spherical Al₂O₃-NPs as 34.5 nm. The hydrodynamic size in Milli Q water and Zeta potential were determined to be 254 nm and +52.2 mV, respectively. The minimal inhibitory concentration of Al₂O₃-NPs was found to be in the range of 1,600–3,200 µg/ml. Treatment at concentrations ≥2,000 µg/ml, resulted in complete growth inhibition of extended-spectrum β-lactamases and metallo-β-lactamases isolates. Scanning electron microscopy analysis revealed the clusters of nanoparticles attached to the bacterial cell surface, causing structural deformities in treated cells. High resolution-transmission electron microscopy analysis confirmed that nanoparticles crossed the cell membrane to become intracellular. The interaction of nanoparticles with the cell membrane eventually triggered the loss of membrane integrity, most likely due to intracellular oxidative stress. The data explicitly suggested that the synthesized Al₂O₃-NPs can be exploited as an effective bactericidal agent against extended-spectrum β-lactamases, non-extended-spectrum β-lactamases and metallo-β-lactamases strains of P. aeruginosa, regardless of their drug resistance patterns and mechanisms. The results elucidated the clinical significance of Al₂O₃-NPs in developing an effective antibacterial therapeutic regimen against the multi-drug resistant bacterial infections. The use of leaf extract of lemongrass for the synthesis of Al₂O₃-NPs appears to be cost effective, nontoxic, eco-friendly and its strong antibacterial activity against multi-drug resistant strains of P. aeruginosa offers compatibility for pharmaceutical and other biomedical applications.

Keywords Green synthesis · Lemongrass · Al₂O₃-NPs · DLS · XRD · Zeta potential · HR-TEM

Introduction

The bacterium Pseudomonas aeruginosa has been considered to be one of the most important pathogens, responsible...
Zinc oxide quantum dots: a potential candidate to detain liver cancer cells

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Abstract  The term cancer is used for diseases in which abnormal cells proliferate without control and are able to attack with other tissues. Over various types of cancers, liver cancer is the most hurtful disease, which affects the whole body system. The aim of the present study was to investigate the efficiency against cancer cells of HepG2 cells, with quantum dots of ZnO. The cytotoxic effects were analyzed with MTT assays in range of 1–100 µg/ml. The cells were exposed to ZnO-QDs and it exhibit significant reduction, which starts from concentration 5 µg/ml (4 %; p < 0.05). The assay was justified with quantitative RT-PCR and it demonstrates, exposure of ZnO-QDs on HepG2 cells. The level of mRNA expressions was significantly up-regulated (Bax, P53, and Caspase-3), whereas the anti-apoptotic gene (Bcl-2) was down-regulated. The QDs (5 ± 2 nm) were prepared via soft chemical solution process and analyzed using FESEM, TEM and HR-TEM.

Keywords  Quantum dots · HepG2 cells · FESEM · TEM · Apoptosis

Introduction

Cancer is not only one disease, its a collection of many disease, which is caused due to cellular disorder. The cellular disorder happens in the body either by natural mutation or through apoptosis. Till date, over more than 100 different types of cancers are observed. Mostly, cancers are named for the organ or type of cell, from where they start for example, cancer that begins in melanocytes of the skin is called melanoma. Over various types of cancers, hepatocellular carcinoma is the fourth most common malignant tumor in the world [1, 2]. Usually, lung cancer is prevalent in many countries such as the United States, United Kingdom, Japan and China. With an estimated average of 0.5–1 million cases diagnosed every year worldwide, it accounts for 5.6 % of all human cancers, with 7.5 % among men and 3.5 % among women. This disease generally affects the developing countries and has a greater share of burden to the leading cause of cancer incidence and mortality among males [3–7]. Hepatocellular carcinoma (HCC) and cholangio carcinoma are the two major types, which accounting for 85 and 10 % of all primary liver cancers, respectively [4–7]. Approximately 81 % of all HCC cases are found in Asia and Africa, with China producing 53 % of these cases. The disease affects at the age of 20 at high risk, whereas typically stabilized at age of 50 and older [4–7]. The most common failure of hepatic is the chronic hepatitis or alcoholic liver disease, which ends in the form of cirrhosis. Mostly (about 85 %), the patients are infected chronically with hepatitis B virus (HBV) which develops cirrhosis [4–8]. Therapies such as, chemotherapy, radiotherapy, immune therapy have been adopted to protect cancer but the outcome rate is remains negligible [9–11]. Recently, the combined efforts of nanotechnology and nano-biotechnology are largely used...
Utilization of photocatalytic ZnO nanoparticles for deactivation of safranine dye and their applications for statistical analysis

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HIGHLIGHTS
- Photocatalysts ZnO-NPs were synthesized via solution process.
- The efficiency of photocatalyst against SA dye was 70.39%.
- Analytical determination was used to validate the photocatalytic study.
- Photocatalysed suspension solution analyzed at low conc. (0.5 – 2.0 μg mL⁻¹).
- The LOD:LOQ limits were 0.060: 0.182 μg mL⁻¹ for nanoparticles.

GRAPHICAL ABSTRACT
The analytical techniques applied to quantify the concentration limit of photocatalysed solution (ZnO), which was highly effective for the deactivation of safranine dye.

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ABSTRACT
A soft chemical solution process was used in synthesis of photocatalytic zinc oxide nanoparticles (ZnO-PNPs) at low temperature. The synthesized PNPs were characterized in terms of their crystallinity, morphological, catalytic, spectroscopic and statistical analysis techniques. X-ray powder diffraction patterns (XRD) were used to know the crystalline property of the prepared materials whereas field emission electronic microscopy (FESEM) was employed to observe the morphology of grown NPs. UV–visible spectroscopy was employed to analyze the absorbance of degraded safranine (SA) dye in presence of NPs at desired time interval. Parameters of statistical analysis give necessary information for established analytical procedures to ensure quality and purity of results. With the help of this analytical method, outcomes were calculated in terms of absorbance such as standard deviation (SD), relative standard deviation (RSD), etc. at 95% confidence level. The photocatalytic deactivation/degradation process significantly enhanced the activity of ZnO-PNPs under UV–visible light in presence of SA dye. The effective concentration of used PNPs was optimized and validated via standard analytical procedure, which exhibited greater significance on deactivation process. The absorption spectra of photocatalyzed solution and activity of ZnO-PNPs were compared with those of pure ZnO, obtained by UV–visible spectroscopy.

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Synthesis and characterization of 2-substituted benzimidazoles and their evaluation as anticancer agent

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Abstract

In this work, we report a series of benzimidazole derivatives synthesized from benzene-1,2-diamine and aryl-aldehydes at room temperature. The synthesized compounds have been characterized on the basis of elemental analysis and various spectroscopic studies viz., IR, 1H- and 13C-NMR, ESI-MS as well by X-ray single X-ray crystallographic study. Interaction of these compounds with CT-DNA has been examined with fluorescence experiments and showed significant binding ability. All the synthesized compounds have been screened for their antitumor activities against various human cancer cell lines viz., Human breast adenocarcinoma cell line (MCF-7), Human leukemia cell line (THP-1), Human prostate cancer cell lines (PC-3) and adenocarcinomic human alveolar basal epithelial cell lines (A-549). Interestingly, all the compounds showed significant anticancer activity.

Introduction

Benzimidazole is a heterocyclic moiety possessing wide spectrum of biological and clinical applications due to their structural resemblance to the naturally occurring nucleotides found in living system [1,2]. Moreover, nucleus of benzimidazole is also found in variety of naturally occurring compounds such as vitamin B12, structurally similar to purine bases. Therefore, benzimidazole and its derivatives represent one of the highly biologically active class of compounds possessing various biological activities [3–7], viz., antimicrobial [8–11], anti-inflammatory [12–15], antiviral [16], anthelmintic [17], anti-tumor [18], anticancer [19], antivirus [20], antihistamine [21], antihypertensive [22], antineoplastic [23], anti-analgesic [24], antiprotozoal [25], anti-hepatitis B virus activity [26]. Furthermore, they also exhibit a remarkable activity against several viruses such as HIV [27], herpes (HSV-1) [28], and RNA influenza [29]. In addition, benzimidazoles serve as ligands for asymmetric catalysts in various reactions [30]. Therefore, the synthesis of these benzimidazole-containing compounds has received considerable attention in diverse areas of chemistry, and a number of synthetic methods have been developed to uncover a variety of new reagents for their preparation [31–40]. The most commonly-used synthetic approach typically involve the

Keywords:
Benzimidazoles
Crystal structure
DNA interactive studies
Anti-proliferative studies
Concentration-Dependent Induction of Reactive Oxygen Species, Cell Cycle Arrest and Apoptosis in Human Liver Cells After Nickel Nanoparticles Exposure

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ABSTRACT:: Due to advent of nanotechnology, nickel nanoparticles (Ni NPs) are increasingly recognized for their utility in various applications including catalysts, sensors and electronics. However, the environmental and human health effects of Ni NPs have not been fully investigated. In this study, we examined toxic effects of Ni NPs in human liver (HepG2) cells. Ni NPs were prepared and characterized by X-ray diffraction, transmission electron microscopy and dynamic light scattering. We observed that Ni NPs (size, ~28 nm; concentration range, 25–100 μg/mL) induced cytotoxicity in HepG2 cells and degree of induction was concentration-dependent. Ni NPs were also found to induce oxidative stress in dose-dependent manner evident by induction of reactive oxygen species and depletion of glutathione. Cell cycle analysis of cells treated with Ni NPs exhibited significant increase of apoptotic cell population in subG1 phase. Ni NPs also induced caspase-3 enzyme activity and apoptotic DNA fragmentation. Upregulation of cell cycle checkpoint gene p53 and bax/bcl-2 ratio with a concomitant loss in mitochondrial membrane potential suggested that Ni NPs induced apoptosis in HepG2 cells was mediated through mitochondrial pathway. This study warrants that applications of Ni NPs should be carefully assessed as to their toxicity to human health.

Keywords: nickel nanoparticles; human liver cells; health effects; oxidative stress; cell cycle

INTRODUCTION

Metallic nickel (Ni) and Ni compounds are released into the atmosphere during mining, smelting, and refining operations representing an environmental and industrial pollutant (Cavallo et al., 2003; Magaye and Zhao, 2012). Evidence
Interactive Effects of Growth Regulators, Carbon Sources, pH on Plant Regeneration and Assessment of Genetic Fidelity Using Single Primer Amplification Reaction (SPARS) Techniques in *Withania somnifera* L.

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**Abstract** An improved and methodical in vitro shoot morphogenic approach through axillary bud multiplication was established in a drug yielding plant, *Withania somnifera* L. Effects of plant growth regulators [6-benzyladenine (BA), kinetin (Kin), 2-isopentenyladenine (2iP), and thidiazuron (TDZ)] either singly or in combination with α-naphthalene acetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA) in Murashige and Skoog (MS) medium were tested. The highest regeneration frequency (90 %) with optimum number of shoots (32±0.00)/explant were obtained on MS medium fortified with 2.5 μM 6-benzyladenine (BA) and 0.5 μM NAA and 30 g/l sucrose at pH 5.8. Among the tried TDZ concentrations, 0.5 μM resulted in maximum number of shoots (20.4±0.40) after 4 weeks of exposure. The proliferating shoot cultures established by repeated subculturing of the mother explants on the hormone-free medium produced the highest shoot number (29.4±0.40) with shoot length (6.80±0.12 cm)/explant at fourth subculture passage, which a decline in shoot proliferation was recorded. Different concentrations of NAA were tested for ex vitro rooting of microshoots. The maximum percentage of rooting 100 % with maximum roots (18.3±0.1) was achieved in soilrite when basal portion of the microshoots were treated with 200 μM (NAA) for 15 min per shoot. The plantlets went through hardening phase in a growth chamber, prior to ex vitro transfer. The PCR-based single primer amplification reaction (SPAR) methods which include random amplified polymorphic DNA (RAPD) and direct amplification of minisatellite DNA (DAMD) markers has been used for assessment of genetic fidelity.
Sub-MICs of *Mentha piperita* essential oil and menthol inhibits AHL mediated quorum sensing and biofilm of Gram-negative bacteria

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Bacterial quorum sensing (QS) is a density dependent communication system that regulates the expression of certain genes including production of virulence factors in many pathogens. Bioactive plant extract/compounds inhibiting QS regulated gene expression may be a potential candidate as antipathogenic drug. In this study anti-QS activity of peppermint (*Mentha piperita*) oil was first tested using the *Chromobacterium violaceum* CVO26 biosensor. Further, the findings of the present investigation revealed that peppermint oil (PMO) at sub-Minimum Inhibitory Concentrations (sub-MICs) strongly interfered with acyl homoserine lactone (AHL) regulated virulence factors and biofilm formation in *Pseudomonas aeruginosa* and *Aeromonas hydrophila*. The result of molecular docking analysis attributed the QS inhibitory activity exhibited by PMO to menthol. Assessment of ability of menthol to interfere with QS systems of various Gram-negative pathogens comprising diverse AHL molecules revealed that it reduced the AHL dependent production of violacein, virulence factors, and biofilm formation indicating broad-spectrum anti-QS activity. Using two *Escherichia coli* biosensors, MG4/pKDT17 and pEAL08-2, we also confirmed that menthol inhibited both the *las* and *pqs* QS systems. Further, findings of the *in vivo* studies with menthol on nematode model *Caenorhabditis elegans* showed significantly enhanced survival of the nematode. Our data identified menthol as a novel broad spectrum QS inhibitor.

Keywords: anti-quorum sensing activity, peppermint oil, menthol, biofilm, molecular docking, *C. elegans*

Introduction

Emergence and spread of antibiotic resistance among pathogenic bacteria represents a major obstacle in treatment of infectious diseases. About 80% of the infections caused by microorganisms are biofilm based (Davies, 2003). Biofilm architecture consists of structured and aggregated communities of bacteria encased in a self-secreted exopolymeric substance (EPS; Costerton et al., 1995). Several studies have revealed that bacteria have developed resistance because of the prolonged treatment with conventional antibiotics possessing a broad-range efficacy via toxic or growth-inhibitory effects on target organisms rendering the traditional antibiotic treatment...
Research Article

Trigonella foenum-graceum (Seed) Extract Interferes with Quorum Sensing Regulated Traits and Biofilm Formation in the Strains of Pseudomonas aeruginosa and Aeromonas hydrophila

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Trigonella foenum-graecum L. (Fenugreek) is an important plant of the Leguminosae family known to have medicinal properties. However, fraction based antiquorumsensing and antibiofilm activities have not been reported from this plant. In the present study T. foenum-graecum seed extract was sequentially fractionated and sub-MICs were tested for above activities. The methanol fraction of the extract demonstrated significant inhibition of AHL regulated virulence factors: protease, LasE elastase, pyocyanin production, chitinase, EPS, and swarming motility in Pseudomonas aeruginosa PAO1 and PAF79. Further, QS dependent virulence factor in the aquatic pathogen Aeromonas hydrophila WAF38 was also reduced. Application of T. foenum-graecum seed extract to PAO1, PAF79, and WAF38 decreased the biofilm forming abilities of the pathogens by significant levels. The extract also exhibited reduced AHL levels and subsequent downregulation of lasB gene. In vivo study showed an enhanced survival of PAO1-preinfected C. elegans after treatment with extract at 1mg/mL. Further, the major compound detected by GC-MS, caffeine, reduced the production of QS regulated virulence factors and biofilm at 200µg/mL concentration indicating its role in the activity of the methanol extract. The results of the present study reveal the potential anti-QS and antibiofilm property of T. foenum-graecum extract and caffeine.

1. Introduction

Formation of biofilm by many pathogens is closely associated with density dependent cell–cell communication known as quorum sensing (QS), in which small diffusible signaling molecules called autoinducers regulate gene expression. Quorum sensing helps bacterial populations to switch from acting as individual cells to operating in a concerted, multicellular fashion [1]. In clinical settings, biofilms are major threat and challenge because bacteria living within the mode are more protected against host immune responses and are significantly more resistant to various antimicrobial drugs [2, 3]. Pseudomonas aeruginosa is an opportunistic, nosocomial, and biofilm forming gram negative pathogen that has three main QS pathways. The rhlI/rhlR and lasI/lasR pathways are (acyl homoserine lactone) AHL based and PQS-MvfR pathway is regulated by 2-heptyl-3-hydroxy-4(1 H)-quinolone signal molecule [4–6]. P. aeruginosa utilizes these signal molecules for the production of biofilms and virulence factors during pathogenesis. Several studies have also shown that QS deficient P. aeruginosa has reduced biofilm forming abilities [7, 8]. The above-mentioned observations imply that
Abstract- The concept of reduction in particle size of metals is recognized since Charak Samhita and confirmed by recent studies by various workers. The herbo-mineral formulations of Ayurveda known as 'Bhasma' are the major source of nanoparticle in Ayurvedic preparation and may be equivalent with nanotechnology witnessing the production of nanoparticles in the contemporary era. In this article, we have discussed the basics of nanoparticle detection and characterization from Bhasma and its potential application. On the other hand, a literature survey was done on the potential of Ayurvedic medicinal plants for their green synthesis of nanoparticles. Thirty-six plants belonging to twenty-seven plant family were demonstrated to synthesize silver and or gold nanoparticle. Based on present literature it can be concluded that Bhasma can be effectively used in enhancing the efficacy of Ayurvedic medicine through nanoparticles contribution. Further interaction of plant extract in contributing green synthesis of nanoparticles needs to be explored in Ayurvedic preparations containing Bhasma.

Keywords: Bhasma, Nanoparticles, Ayurvedic plants

Introduction

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. Nanoparticles provide detail insight understanding the degree and variation in physiochemical properties of a voluminous material and its structures at a molecular level. Particle size often plays the insignificant role in determining the physical properties of a mass material, whereas, in the case of nanoscale material, this is not true, rather at nano levels, these same particles exhibit some of the most interesting and peculiar characteristics (Kumar et al., 2006). This variation or acquired characteristics of the nanoparticle is attributed to its very high aspect ratio, along with other explanations. This very high aspect ratio tremendously increases the biological efficacy of nanoparticles. Interaction of nanoparticle with other particles or structure also increases significantly, another important trait that explains the enhanced biological activity of nanoparticles, also attributed to its extraordinary aspect ratio. This concept of reduction of particle size of metal is prevailing since Charka Samhita. For a metallic preparation of metal based formulations, the metal is heated to very high temperature, and flaks are quenched in liquid media until a fine powder of metal is form. Nanotechnology can work at this level, to form the larger bioactive entity with a new structure of different physiochemical properties. Bhasmas are unique metallo-herbal Ayurvedic preparations are commonly suggested for treatment of multiple ailments also with other Ayurvedic medicines (Sarkar and Chaudhary, 2010).

In recent years, there is a tremendous interest shown by the scientific community in nanoparticles and development of nanotechnology and nanomedicine with great expectation and potential applications in health and medicine. Research in the traditional medicine has also been accelerated in the recent past due to its global recognition. The role of nanoparticles in Ayurvedic system of medicine was recognized since long. However, scientific evaluation and scientific data are less known. On the other hand, the use of metals and its oxides in traditional medicine is often considered a matter of toxicity points of view as Bhasma contains...
TLC and Spectroscopic Based Determination of Free Radical Scavenging Activity of Seven Ayurvedic Medicinal Plants

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Abstract- Antioxidant activity of medicinal plants is widely recognized for their therapeutic value. Various in-vitro and in-vivo methods are used to assess antioxidants in plant extracts. However, in the screening of large number of plants, rapid method of detection of active compounds is required to develop further activity guided fractionation of the extracts. In this study, methanolic crude extracts of seven plants namely Balsamodendron mukul, Boerhavia diffusa, Catharanthus roseus, Curcuma longa, Lawsonia inermis, Laurus nobilis and Piper nigrum were subjected to phytochemical analysis by color test and TLC for detection of phytocompounds, particularly flavonoids. DPPH bio-autographic method was used to detect the antioxidant activity of particular spot on the pre developed TLC plate. Further each extract was also assessed by standard spectroscopic analysis for DPPH radical scavenging potential. The data obtained is comparable and highlights the importance of TLC based method for fast detection of antioxidant active phytocompounds in the crude extract of the plant/Ayurvedic drugs.

Key words: Antioxidant activity, Medicinal plant, Phytocompound, TLC.

Introduction  
Free radical facilitated cell damage plays an important role in the development of numerous human diseases. It has been widely assumed that progression of diseases such as cardiovascular disease, neuronal disease, cataracts, and several types of cancer are mediated with free radicals generation that ultimately leads to the onset of oxidative stress (Thomas & Kalyanaraman, 1997). Recent clinical studies have indicated positive role of natural antioxidant in significant reduction in the risk of coronary heart disease and ageing related disease (Valko et al., 2007). On the other hand, certain toxicity potential and carcinogenicity of synthetic antioxidants such as BHA and BHT have been reported (Sasaki et al., 2002). Therefore, there is an increasing interest in search of natural antioxidant from plants such as vegetables, fruits and medicinal herbs. Antioxidant activity in plants is very commonly reported but it is challenging to rapidly screen the most active antioxidants in the plant extracts due to its complex nature and presence of large number of interfering constituents. Numerous methods have been developed for screening of plant extracts for their antioxidant potential, among these TLC-bio-autography method (Cimpoiu, 2006) can quickly detect and separate the antioxidant active constituents from complex crude extracts (Gu et al., 2009).

Various plants used in the traditional medicinal system produce a lot of antioxidants and represent rich source of new and comparatively nontoxic phytocompounds with antioxidant activity. It has been widely accepted that antioxidant active secondary metabolites including simple phenolic acids to highly polymerized tannins impart therapeutic potential to various plants (Pandey and Rizvi, 2009). Moreover, majority of plants derived antioxidant active compounds falls in the category of polyphenolics (e.g. phenolic acids, flavonoids, and tannins) and among these flavonoids represent most versatile group in terms of their antioxidant and other therapeutic values (Cimpoiu, 2006). Depending upon the chemical modification in the basic benzo-γ-pyran structure, flavonoids chemically subdivided into flavonols, flavones, isoflavones, flavanones and anthocyanidins (Harborne and Baxter, 1993). Characterization of these biologically active phytochemicals present in the plant along with the
Single and Multi-Component Adsorption of Metal Ions by Acinetobacter sp. FM4

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This paper reports biosorption of Cr(VI), Cu(II), and Ni(II) onto Acinetobacter sp. FM4 biomass isolated from soil irrigated with tannery effluent from single, binary, and ternary metal solutions. Optimum pH for biosorption was found to be 2.0 for Cr(VI), 5.0 for Cu(II), and 6.0 for Ni(II) ions. Sorption capacities for Cr(VI), Cu(II), and Ni(II) ions were estimated as 90 mg g⁻¹, 93.3 mg g⁻¹, and 66.7 mg g⁻¹, respectively. The combined effect of adsorbing one metal ion in the presence of another metal ion reduced the adsorption capacity of either metal ion. The presence of functional groups on the cell wall surface of the biomass that may interact with the metal ion was confirmed by Fourier Transform Infrared (FTIR) spectroscopy.

Keywords Acinetobacter; biosorption; multimetal; isotherms

INTRODUCTION

Pollution from heavy metals has become a serious problem for human health and for the environment. The existence of heavy metals, such as copper (Cu), nickel (Ni), zinc (Zn), lead (Pb), mercury (Hg), chromium (Cr), and cadmium (Cd) in wastewater is the consequence of several activities like chemical manufacturing, paint pigments, plastics, tanning, metallurgy, and the nuclear industry (1). Heavy metals are persistent environmental contaminants since they cannot be degraded or destroyed (2). Heavy metal pollution represents an important problem due to its toxic effect and accumulation throughout the food chain which leads to serious ecological and health problems (3). Removal and recovery of heavy metals are very important with respect to environmental and economical considerations. Conventional physicochemical methods such as electrochemical treatment, ion-exchange, precipitation, reverse osmosis, evaporation, and oxidation/reduction for heavy metal removal from waste streams are expensive, not eco-friendly (4), and inefficient for metal removal from diluted solutions containing from 1 to 100 mg L⁻¹ of the dissolved metal (3). Therefore, there is a need for the development of economical, effective, and safe methods for removal of toxic metals.

Biosorption is relatively a new treatment methodology for removing heavy metal ions from dilute solutions. Biosorption has received considerable attention owing to the fact that it is an efficient, clean, and cheap technology for the treatment of wastewater. A lot of research has been carried out for developing and employing inexpensive biosorbents for the treatment of wastewaters containing heavy metals (4, 5). More recently, attention has been focused on the use of microbial biomass particularly bacteria for the removal of heavy metals from aqueous solutions (6, 7).

Most of the biosorption studies have been focused on single metal systems (8, 9). In contrast, relatively little work has been contributed to elucidate the biosorption behavior in multi-metal systems (9). When more than one metal is present in a sorption system, evaluation, interpretation, and representation of biosorption results become very complicated. Thus, a sorption research for multiple metals is more realistic than for a single metal system. In multi-component systems, the sorption effect of heavy metal depends on surface properties of adsorbents, temperature, pH, initial metal ion concentration, dosage, and the metallic species competing for binding sites (10). The composition of a metal solution also deeply affects metal retention onto solid surfaces.

In the present study, Acinetobacter sp. isolated from soil irrigated with tannery effluents was tested to evaluate its competence as a biosorbent for the removal of heavy metal ions from aqueous solutions. The influence of various process parameters such as pH, contact time, and initial metal ion concentration was studied. The Langmuir and Freundlich adsorption models were employed for mathematical description of experimental equilibrium data. Furthermore, the sorption dynamics of Cr(VI), Cu(II), and Ni(II) ions during their biosorption by the bacterial strain from single, binary, and ternary systems were evaluated. Besides, the bacterium was applied to real tannery effluents to validate its effectiveness in true processing.
PRODUCTION OF PLANT-GROWTH PROMOTING SUBSTANCES BY NODULE FORMING SYMBIOTIC BACTERIUM RHIZOBIUM SP. OS1 IS INFLUENCED BY CuO, ZnO AND Fe₂O₃ NANOPARTICLES

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ABSTRACT

Symbiotic nitrogen fixing rhizobia besides fixing atmospheric nitrogen also produces plant growth promoting substances such as indole acetic acids, siderophores, and cyanogenic compounds etc. However, the effects of nanomaterials on plant growth regulating substances synthesized by these bacteria are not reported. In this paper we have examined the impact of varying concentration of three metal oxide nanoparticles (MONPs) namely copper oxide (CuO), iron oxide (Fe₂O₃) and zinc oxide (ZnO) on growth behaviour and plant growth promoting activities of nodule forming bacterium Rhizobium sp. strain OS1. The three MONPs tested in this study differentially affected the levels of plant growth regulating substances in a dose dependent manner which varied with species of each nanoparticle. A maximum reduction in indole acetic acid, hydrogen cyanide, ammonia and siderophores, expressed by Rhizobium sp. OS1 was observed at 150 µg/ml each of CuO, Fe₂O₃ and ZnO. Iron oxide did not show any toxicity to siderophores. At 50 µg/ml, CuO induced the IAA production by 11% which decreased progressively with increasing concentrations. The synthesis of HCN and NH₃ was completely abolished when strain OS1 was grown with 150 µg/ml of all nanoparticles. Unlike plant growth promoting substances, the production of exo-polysaccharide increased gradually with increasing concentration of each MONPs by rhizobial strain. This study suggests that the nanoparticles of different functional groups affect the physiological expression of rhizobial species differently and it further opens up a new vistas to better understand the impact of nanoparticles on symbiotic interaction between rhizobia and legumes.

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[1] INTRODUCTION

Recently, nanoscience has become one of the most promising fields of research with greater impact on economy and environment health. The research on nanomaterials: materials of 100 nm in at least one dimension, is likely to result in the production of huge number of new nano-products in the coming years. Considering the importance of nanotechnology, a greater attention has been paid on this industry which is expected to reach a market size of approximately 2.6 trillion dollars by 2015 [1]. In addition, nanotechnology is also likely to influence agricultural research especially in (i) the conversion of agricultural and food wastes to energy and other useful by-products through enzymatic nano-bio-processing (ii) disease prevention and treatment of plants using various nanomaterials [2] and (iii) reproductive science and technology. Despite these benefits, the increasing numbers of commercial products, from cosmetics to medicine and fertilizers to crop products are adding sufficient amounts of nanomaterials ultimately to soils. Such nanoparticles have however, been found highly resistant to degradation and persist in soil or water bodies. Nanomaterials for example carbon nanotubes [3, 4], graphene-based nanomaterials [5], iron-based nanoparticles [6], silver [7] and copper, zinc and titanium oxide nanoparticles [8, 9] have been reported to cause biologically undesirable toxic effects on both deleterious (DRMOs) and beneficial rhizosphere microorganisms [10-12] including Escherichia coli, Bacillus subtilis, and Streptococcus aureus [13]. Pseudomonas chlororaphis [14-18], Pseudomonas putida [11] and Campylobacter jejuni [19]. However, the reports on the effect of nanoparticles on secondary metabolites of microbes are conflicting. For example, Dimpka et al. [16] in a recent study found that sub-lethal levels of CuONPs reduced the secretion of plant growth promoting substance siderophore in P. chlororaphis O6 whereas ZnO NPs increased the production of the fluorescent siderophore pyoverdine. Similarly, a contrasting effect of CuO and ZnO NPs on siderophores and IAA has also been reported by Dimpka et al. [18] suggesting that the effect of NPs on secondary metabolite production by bacterial populations cannot be generalized rather it is highly
Screening of Multiple Metal and Antibiotic Resistant Isolates and Their Plant Growth Promoting Activity

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Abstract: Heavy metal contamination has accelerated due to the rapid industrialization world wide. Accumulation of metals in excess can modify the structure of essential protein or can replace an essential element. Bradyrhizobium strains showed tolerance to cadmium, chromium, nickel, lead, zinc and copper. All the isolates showed maximum tolerance towards lead and zinc which was followed by nickel and chromium. These strains also showed tolerance towards most of the antibiotics. Bradyrhizobium strains were also tested for their Plant Growth Promoting (PGP) substances, all isolates produced good amount of indole acetic acid and were positive for ammonia but only three strains were positive for HCN and siderophore (RM1, RM2 and RM8), the rest isolates showed negative result. Based on the above intrinsic abilities of Bradyrhizobium species, these strains can be used for the growth promotion, as well for the detoxification of the heavy metals in metal polluted soils.

Key words: Bradyrhizobium, heavy metal tolerance, antibiotic resistance, PGP activities

INTRODUCTION

Heavy metals and metalloids are major global environmental pollutants and many of them are toxic even at very low concentrations. Such trace elements are released into the biosphere from different industries which use them frequently for manufacturing various products (Fernandes and Henrique, 1991). The industries using Heavy Metals (HM) include mining, smelting, manufacturing, gas exhaust, energy and fuel production, fertilizer, sewage and pesticide production and municipal waste generation. After discharge, heavy metals are reported to adversely affect about 12% of the world’s agricultural land (Moffat, 1999). According to some estimates, metal concentrations in soil range from less than 1 mg kg⁻¹ to as high as 100,000 mg kg⁻¹, which could either be geological in origin or may result from different human activities (Blaylock and Huang, 2000). Even though some heavy metals are required by plants to maintain its physiological processes but the excessive accumulation of such metals in plant tissues have shown toxicity symptoms and have often been found lethal as they- (1) can modify structure of some essential proteins (Yuelu, 2005; Roy et al., 2010) (2) can replace certain elements able to cause chlorosis (Ebbas and Uchil, 2008), growth inhibition, structure damage, browning of roots (Roy et al., 2010) and (3) decline in physiological and biochemical activities including inhibition of photosynthesis (Cheng, 2003; Gorhe and Paszkowski, 2006; Wani et al., 2007a). The threat by heavy metals to plants and consequently to human and other animals is aggravated by low mobility and their ability to persist in the environment. For instance, lead (Pb), one of the more persistent metals was estimated to have a soil retention time of 150 to 5000 years while the average biological half-life of Cd has been reported as 18 years.

Soils heavily contaminated with several toxic metals, can adversely affect not only beneficial rhizospheric microbes but also plant growth at elevated concentrations (Giller et al., 1998; Rajkumar et al., 2006). Metal toxicity, however, can be reduced by applying resistant microorganisms (Wani et al., 2009). Generally, the Plant Growth Promoting Rhizobacterial (PGPR) strains can promote plant growth and through phosphate solubilization, IAA and synthesis of antimicrobial compounds and siderophores when these strains are applied to seeds or to soils (Wasi et al., 2008). Moreover, metal-resistant microbes can detoxify metals either by enzymatically or through the production of metabolites, through accumulation and sequestration of the metal ions.
Toxicity, PGP Activity, Bioaccumulation of Cadmium, Copper and Chromium (VI) in Nitrogen-fixing Rhizobacteria

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ABSTRACT

Heavy metal pollution of soil is a significant environmental problem and has its negative impact on human health and agriculture. Rhizobial isolates present in rhizospheric region can play a key role in remediation of polluted sites, in which, microbial populations are known to affect heavy metal mobility and availability to the plant through release of indole acetic acid, siderophores, ammonia, phosphate solubilizing agents and other Plant Growth Promoting (PGP) substances and therefore, have potential to enhance bioremediation processes. Bioremediation strategies with suitable heavy metal-adapted rhizobacteria have received a lot of attention. This study tells the effect of heavy metals (Cd²⁺, Cu²⁺ and Cr⁶⁺) on the growth of different rhizobial cultures, their PGP producing ability and significance of bioaccumulation of toxic heavy metals on rhizobium.

Key words: Rhizobium, rhizobacteria, plant growth promoting activity, heavy metals, bioaccumulation

INTRODUCTION

With the rapid industrialization and development of the agricultural practices, heavy metal pollution in soil has increasingly becomes a serious threat to the environment. Giller et al. (1998) reported that metal-polluted environment poses a damaging effect to soil microbial diversity and microbial activities (indices of microbial metabolism and of soil fertility). Their accumulation on the culturable layer of soil changes the trace element profile and thereby causing physiological and genetic changes to various lives (Mudakavi and Narayana, 1997; Chhonkar et al., 2006). Different techniques developed so far for the metal removal, are quite expensive and requires use of contaminating product for desorption of metals and for cleaning up of inorganic matrix. Removal of toxic heavy metal from the soils is of great importance not only because of the decontamination effect but also because this removal protect plants from the effect of toxic metal and ensures the functioning of plants. Metal accumulation is an alternative mechanism for metal detoxification in bacteria (Gadd, 1990). Bacteria have evolved several types of mechanisms to tolerate the heavy metal ions. These include reduction of the heavy metal ions to a less toxic state (Nies, 1999), the
Synthesis, characterization and toxicological evaluation of iron oxide nanoparticles in human lung alveolar epithelial cells

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ABSTRACT

The present investigation was aimed to characterize the synthesized iron oxide nanoparticles (Fe3O4-NPs) and to assess their cytotoxicity and oxidative stress in human lung alveolar epithelial cells (A-549). Fe3O4-NPs were characterized by X-ray diffraction, transmission electron microscopy, dynamic light scattering, and atomic force microscopy. The morphology of the Fe3O4-NPs was found to be variable with a size range of 36 nm. A-549 cells were exposed to Fe3O4-NPs (10–50 μg/ml concentrations) for 24 h. Post exposure, cytotoxicity assays (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT; neutral red uptake, NRU; and cellular morphology) and oxidative stress (lipid peroxidation, LPO and glutathione, GSH) were evaluated. Further, intracellular reactive oxygen species (ROS) generation and mitochondrial membrane potential (MMP) were also studied. MTT and NRU assays revealed a concentration-dependent decrease in the cell viability of A-549 cells. Fe3O4-NPs exposed cells also altered the normal morphology of the cells. Furthermore, the cells showed significant induction of oxidative stress. This was confirmed by the increase in LPO and ROS generation, and the decrease in the GSH level and MMP. Our results demonstrated that Fe3O4-NPs induced cytotoxicity is likely to be mediated through the oxidative stress and ROS generation in A-549 cells.

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1. Introduction

Metal oxide nanoparticles are widely being used in many industrial products, i.e., catalysts, pigments, food additives, sun screens and cosmetics [1,2]. The applications and productions of these nanoparticles at large number have brought attention to their risk factors. It is well known that nanoparticles are being released during particle synthesis and handling of dry powders and liquid suspensions [3]. Toxicity of various metal oxide nanoparticles has also been reported in vitro [4–9] as well as in vivo [10–12] setups. Numerous studies indicate that metal oxide nanoparticles have the ability to generate reactive oxygen species (ROS) [13,14] and they are involved in the cytotoxicity due to their small size and large surface area [15]. Experimental evidences also showed that nanoparticles released by sprays and powders can potentially deposit in the respiratory system [16,17].

Iron oxide (Fe3O4) nanoparticles have been applied broadly to bioscience and clinical research for various purposes; the most common includes magnetic cell labeling [18,19], separation and tracking [20], for therapeutic purposes in hyperthermia [21], in drug delivery [22], and for diagnostic purposes, e.g., as contrast agents for magnetic resonance imaging (MRI) [23]. Although the cytotoxic effects of iron oxide nanoparticles are known [24–26], the mechanism(s) of their induced cytotoxicity is not clearly understood. Since, human exposure to iron oxide may occur through the exposure routes of inhalation and ingestion at occupational settings. Therefore, the present investigation was aimed to understand the mechanism(s) of cell death induced by iron oxide nanoparticles in human lung alveolar epithelial cells (A-549) under in vitro conditions. These in vitro systems are cost-effective, rapid and reproducible with low or no ethical dubious [27,28]. In this study, firstly we have focused on the detailed synthesis and physicochemical characteristics of iron oxide nanoparticles; secondly, the effect
Optical Analysis of Zinc Oxide Quantum Dots with Bovine Serum Albumin and Bovine Hemoglobin

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Abstract Quantum dots (QDs) are widely used in medical, industrial, and household applications owing to their excellent biological property. For its wide medical application, the biocompatibility of QDs is an important aspect of research. The aim of the present study was to synthesize zinc oxide quantum dots (ZnO-QDs) and to investigate the interaction with bovine serum albumin (BSA) and bovine hemoglobin (BHb) using fluorescence quenching method and circular dichroism (CD). The study suggests that the electrostatic force of attraction favors the adsorption of BSA onto ZnO-QDs. The fluorescence quenching of BSA and BHb using QD indicates the formation of QDs-BSA complexes. The CD spectra also showed the changes in secondary structure of proteins by interacting with QDs.

Keywords Quantum dots · Bovine serum albumin · Bovine hemoglobin · Circular dichroism

Introduction

Nanoscale materials have a small diameter, and thus exhibit high surface area and are used for biological and medical systems, including cancer therapies, photodynamic therapy, bio-imaging, and pharmacokinetics studies [1–3]. The applications of nanobiotechnology commence from the attachment of nanoparticles onto targeting cells. Thus, there is an increased interest in understanding the interactions between nanomaterials and biological molecules because of their unique physicochemical properties. Among various nanomaterials, semiconductor zinc oxide (ZnO) has an excellent property and exhibits vast applications in various fields [4]. Given their small size, they can be easily conjugated with DNA [5]. In this manuscript, we have targeted the bovine serum albumin (BSA) and bovine hemoglobin (BHb) because of their known detailed structure and multiple functions. Furthermore, their transporting function made them more interesting for drug development. BSA is a model protein with stability, water solubility, and unusual binding capacity [6] and has structural homology with human serum albumin (HSA). BSA has two tryptophan residues that possess intrinsic fluorescence. Trp-212 locates within a hydrophobic binding pocket in the subdomain IIA, and Trp-134, locates on the surface of the albumin molecule in domain I. On the contrary, BHb is well known for its function in the vascular system of animals, being a carrier of oxygen. It also transports CO₂ and regulates the pH of blood [7]. In view of this, the present study was designed to investigate the synthesis and binding studies of zinc oxide quantum dots (ZnO-QDs) with BSA and BHb using fluorescence and circular dichroism (CD) techniques.
Reactive Oxygen Species Mediated Bacterial Biofilm Inhibition via Zinc Oxide Nanoparticles and Their Statistical Determination

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Abstract

The formation of bacterial biofilm is a major challenge in clinical applications. The main aim of this study is to describe the synthesis, characterization and biocidal potential of zinc oxide nanoparticles (NPs) against bacterial strain Pseudomonas aeruginosa. These nanoparticles were synthesized via soft chemical solution process in a very short time and their structural properties have been investigated in detail by using X-ray diffraction and transmission electron microscopy measurements. In this work, the potential of synthesized ZnO-NPs (~10–15 nm) has been assessed in vitro inhibition of bacteria and the formation of their biofilms was observed using the tissue culture plate assays. The crystal violet staining on biofilm formation and its optical density revealed the effect on biofilm inhibition. The NPs at a concentration of 100 μg/mL significantly inhibited the growth of bacteria and biofilm formation. The biofilm inhibition by ZnO-NPs was also confirmed via bio-transmission electron microscopy (Bio-TEM). The Bio-TEM analysis of ZnO-NPs treated bacteria confirmed the deformation and damage of cells. The bacterial growth in presence of NPs concluded the bactericidal ability of NPs in a concentration dependent manner. It has been speculated that the antibacterial activity of NPs as a surface coating material, could be a feasible approach for controlling the pathogens. Additionally, the obtained bacterial solution data is also in agreement with the results from statistical analytical methods.

Introduction

Increased resistance of bacteria against antibiotic medicines is a global health concern. Bacteria are shown to develop resistance to a majority of commercially available antibiotics. Some bacteria produce slime, which is responsible for bacterial adhesion and formation of biofilms on artificial surfaces. Most of the wound infections often including the Gram-positive (++) Staphylococcus aureus, S.epidermidis, and Gram-negative (–) Pseudomonas aeruginosa [1]. The pathogen Pseudomonas aeruginosa is also known for producing secondary metabolite [2]. These organisms are found to exhibit quorum sensing and produce strong biofilms. The biofilms are surface attached microbial communities embedded in their own microbial-originated matrix of protective and adhesive extracellular polymeric substances (EPSs), mainly polysaccharides, lipids and proteins resistant to antimicrobials [3]. The upcoming approach towards control of biofilms formation involves nanomaterials, which inhibit bacterial adhesion and biofilm formation. NPs with biocidal properties are emerging as new and promising antimicrobial agents as bacteria are less likely to develop resistance against metal NPs than conventional antibiotics. NPs can serve as effective bactericidal materials [4–5] and antimicrobial activity of Al2O3, Fe2O3, CoO2, ZrO2 and MgO against pathogenic microorganism (Pseudomonas sp., Enterobacter sp., Klebsiella sp., morganii and S. aureus) has already been tested by Ravikumar et al., [6]. In another study the effect of silver NPs against water borne pathogens namely Pseudomonas aeruginosa and Vibrio cholerae was tested [7]. Also, the effect of ZnO, CuO, Ag, Au and Bi on dental caries causing bacteria S.mutans has been widely studied [8–10]. The inherent property of bactericidal activity of NPs has prompted us to investigate the role of ZnO-NPs as an effective surface coating antimicrobial agent. Among various metallic and metal oxide nano- and microstructures, zinc ions (Zn²⁺) of zinc oxide has potential to interact with protein, free ions (Zn²⁺) and can also be an effective target in HSV-1 pathogenesis. The tetrapod like structures of ZnO synthesized by flame transport synthesis process capacity to block the entry and spread of HSV-2 virus into target cells and have ability to neutralize HSV-2 virions [11–13]. Towards this direction, several instrumentation and methods have been applied to observe the accuracy and reliability of bacterial strain solution result such as inductively coupled plasma atomic emission spectrometry (ICP-ES), photolumines-
Statistical analysis of gold nanoparticle-induced oxidative stress and apoptosis in myoblast (C2C12) cells

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ABSTRACT

Nanoscale gold particles (Au-NPs) with a diameter below 20 nm are notably important candidates for various important applications because of their extraordinary quantum size effects. Their high surface area-to-volume ratio facilitates their very high reactivities; therefore, they can be utilised in different ways in biomedical applications. For example, these nanoparticles can penetrate into cells and bind with proteins or DNA and are therefore potential nanostructures employed for sensing and detecting various biological identities. In the present work, we synthesised Au-NPs via a colloidal process using chloroauric acid (HAuCl4·4H2O) and trisodium citrate dihydrate (Na3C6H5O7·2H2O) as a reducing agent. The shape evolution and the structural properties of these NPs were investigated in detail using TEM and high resolution HR-TEM investigations. Different doses of Au NPs have been applied to treat C2C12 myoblast cells in a 24-h incubation period, and a dose-dependent study has also been performed. The cells were cultivated in DMEM with FBS and antibiotics (strepto-penicillin) at 37 °C in a 5% humidified environment of CO2 and 95% air. Cell viability analysis using MTT assays revealed that increased concentration of Au NPs (100–1000 ng/mL) resulted in a decreased density of cells. The amount of reactive oxygen species (ROS) in C2C12 cells analysed with Au-NPs (in a dose-dependent manner), and the RT-PCR data demonstrated the up-regulation of caspase-3 and caspase-7 genes in C2C12 cells after treatment with Au-NPs. These results have been confirmed by detailed confocal microscopy (CLSM) studies. In addition, the quantitative analysis of the Au-NPs was also confirmed by statistical analytical parameters, such as precision, accuracy, linearity, limits of detection (LOD) and limit of quantitation (LOQ), quantitative recoveries and relative standard deviation (RSD), and the analyses again exhibited a significant and large effect of Au NPs on C2C12 cells.

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1. Introduction

The treatment of cancer relies on chemotherapeutic, surgical and anticancer drugs [1,2], but all of these approaches are highly expensive and tedious; therefore, this field is urgently seeking better and more reliable therapeutic techniques. In this regard, the combined efforts of nanomaterials and biotechnology offer new and efficient alternatives to treat and diagnose different types of cancers [3–6]. Nanostructures from inorganic materials have been demonstrated to be highly attractive candidates because of their exceptional physical and chemical properties along with their capability to target specific sites and minimise severe side-effects [7–14]. Among the various metals and metal oxide nanostructures, gold NPs are probably the most utilised structures in biomedical engineering, and they have demonstrated potential for various applications, such as tooth implants [15], cancer treatment [16], biomolecular [17], protein folding [18], detection of DNA [19], intracellular labelling [20], drug delivery [21], cancer targeting [22], and imaging [23]. Several reports have also shown that the nanoscale

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ZnO nanoparticles induced oxidative stress and apoptosis in HepG2 and MCF-7 cancer cells and their antibacterial activity

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A B S T R A C T

Liver and breast cancer are the most traumatic diseases because they affect the major organs of the body. Nanomedicine recently emerged as a better option for treatment of these deadly diseases. As a result, many nanoparticles have been used to treat cancer cell lines. Of the various nanoparticles, zinc oxide exhibits biocompatibility. Therefore, the aim of the present study was to investigate the activity of zinc oxide nanoparticles (ZnO-NPs) against HepG2 and MCF-7 cells. The NPs (∼13 ± 2 nm) were prepared via a non-protonated chemical route and were well-characterized through standard techniques. The study showed that treatment with NPs is notably effective against the proliferation of HepG2 and MCF-7 cancer cells in a dose-dependent manner. The MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide, a tetrazole) assays revealed the concentration-dependent cytotoxic effects of NPs in range of 2.5–100 μg/ml. HepG2 and MCF-7 cells were exposed to ZnO-NPs and exhibited a significant reduction in their cell viability (95% and 96%; p < 0.05) in response to a very low concentration (25 μg/ml) of the ZnO-NPs; this finding was confirmed with FACS (fluorescence-activated cell sorting) data. The reduction in cell viability in response to NP treatment induces cytotoxicity in the cultured cells. The quantitative RT-PCR (real-time polymerase chain reaction) results demonstrate that the exposure of HepG2 cells to ZnO-NPs results in significant upregulation of the mRNA expression level of Bax, p53, and caspase-3 and the down regulation of the anti-apoptotic gene Bcl-2. The NPs were also tested against five pathogenic bacteria through the disk diffusion method, and their antibacterial activities were compared with that of ZnO salt.

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1. Introduction

Nanobiotechnology/bionanotechnology, which is the fastest emerging field of material science, connects several branches of basic sciences, including biology, chemistry, biotechnology, mathematics material science, and engineering. The technology develops nanofabricated materials with a very small diameter (1–100) to manipulate and transform various types of biological systems [1–9]. Bionanotechnology exhibits vast applications because it is largely used in cosmetics, skin care products, drug delivery, nanomedicines, cancers, molecular biology, markers, tissue engineering products, non-viral gene carriers, clinical bio-analytical diagnostics, and therapeutics because chemotherapy, radiation, and surgery were the old techniques used to reduce cancers [1–10]. Of the wide range of applications of nanobiotechnology, it is widely applied to reduce cancers with the use of inexpensive inorganic nano-scale materials. Nanomaterials, which have very small diameter (∼30 nm), exhibit unique physicochemical characteristic due to their size; these include a high surface area, low cost, enhanced reactivity, ability to easily enter cells, and ability to affect various types of biological systems [1–12]. It is known that cancer is a heterogeneous and complex disease that occurs when the normal cell proliferation controls are lost. The positive/negative cells change into cancer cells through three distinct phases, i.e., initiation, promotion, and progression. Several reports have reported the toxicity of nanostructures, which kill human cancerous cells and are very useful for protection against cancers [13–15]. Of the various types of cancers, hepatocellular carcinoma is the fourth most common malignant tumour in the world [16,17]. The incidence

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Research Paper

Gum arabic capped-silver nanoparticles inhibit biofilm formation by multi-drug resistant strains of *Pseudomonas aeruginosa*

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Clinical isolates (n=55) of *Pseudomonas aeruginosa* were screened for the extended spectrum β-lactamases and metallo-β-lactamases activities and biofilm forming capability. The aim of the study was to demonstrate the antibiofilm efficacy of gum arabic capped-silver nanoparticles (GA-AgNPs) against the multi-drug resistant (MDR) biofilm forming *P. aeruginosa*. The GA-AgNPs were characterized by UV-spectroscopy, X-ray diffraction, and high resolution-transmission electron microscopy analysis. The isolates were screened for their biofilm forming ability, using the Congo red agar, tube method and tissue culture plate assays. The biofilm forming ability was further validated and its inhibition by GA-AgNPs was demonstrated by performing the scanning electron microscopy (SEM) and confocal laser scanning microscopy. SEM analysis of GA-AgNPs treated bacteria revealed severely deformed and damaged cells. Double fluorescent staining with propidium iodide and concanavalin A-fluorescein isothiocyanate concurrently detected the bacterial cells and exopolysaccharides (EPS) matrix. The CLSM results exhibited the GA-AgNPs concentration dependent inhibition of bacterial growth and EPS matrix of the biofilm colonizers on the surface of plastic catheters. Treatment of catheters with GA-AgNPs at 50 μg ml⁻¹ has resulted in 95% inhibition of bacterial colonization. This study elucidated the significance of GA-AgNPs, as the next generation antimicrobials, in protection against the biofilm mediated infections caused by MDR *P. aeruginosa*. It is suggested that application of GA-AgNPs, as a surface coating material for dispensing antibacterial attributes to surgical implants and implements, could be a viable approach for controlling MDR pathogens after adequate validations in clinical settings.

**Abbreviations**: EPS – exopolysaccharides; GA-AgNPs – gum arabic-silver nanoparticles; CLSM – confocal laser scanning microscopy; SEM – scanning electron microscopy; HR-TEM – high resolution-transmission electron microscopy; ESBL – extended spectrum β-lactamases; MBL – metallo-β-lactamases; ConA-FITC – concanavalin A-fluorescein isothiocyanate; PI – propidium iodide; XRD – X-ray diffraction; MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration; CRA – Congo red agar; TM – tube method; TCP – tissue culture plate

**Keywords**: GA-AgNPs / EPS / CLSM / SEM / ESBL / MBL / ConA-FITC

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**Introduction**

Increased resistance of bacteria to antibiotic therapy is an emerging global health concern. In the last few decades, bacteria have shown to develop resistance to a majority of commercially available antibiotics, whereas the number
Nigella Sativa Seed Extracts Against Lung Cancer Cells

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Abstract

Nigella sativa (N sativa), commonly known as black seed, has been used in traditional medicine to treat many diseases. The antioxidant, anti-inflammatory, and antibacterial activities of N sativa extracts are well known. Therefore, the present study was designed to investigate the anticancer activity of seed extract (NSE) and seed oil (NSO) of N sativa against a human lung cancer cell line. Cells were exposed to 0.01 to 1 mg/ml of NSE and NSO for 24 h, then percent cell viability was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-biphenyl tetrazolium bromide (MTT) and neutral red uptake (NRU) assays, and cellular morphology by phase contrast inverted microscopy. The results showed NSE and NSO significantly reduce the cell viability and alter the cellular morphology of A-549 cells in a concentration dependent manner. The percent cell viability was recorded as 75%, 50%, and 26% at 0.25, 0.5, and 1 mg/ml of NSE by MTT assay and 73%, 48%, and 23% at 0.25, 0.5, and 1 mg/ml of NSE by NRU assay. Exposure to NSO concentrations of 0.1 mg/ml and above for 24 h was also found to be cytotoxic. The decrease in cell viability at 0.1, 0.25, 0.5, and 1 mg/ml of NSO was recorded to be 89%, 52%, 41%, and 13% by MTT assay and 85%, 52%, 38%, and 11% by NRU assay, respectively. A-549 cells exposed to 0.25, 0.5 and 1 mg/ml of NSE and NSO lost their typical morphology and appeared smaller in size. The data revealed that the treatment of seed extract (NSE) and seed oil (NSO) of Nigella sativa significantly reduce viability of human lung cancer cells.

Keywords: Nigella sativa - A-549 cells – cytotoxicity - cellular morphology

Cytotoxicity of Nigella Sativa Seed Oil and Extract Against Human Lung Cancer Cell Line

Introduction

Nigella sativa (N sativa) is an annual herb of the Ranunculaceae family, which is used as an important nutritional flavoring agent and natural health remedy in traditional folk medicine for the treatment of numerous disorders in ancient systems of Unani, Ayurveda, Chinese and Arabic medicine for thousands of years (Randhawa and Alghamdi, 2011). The extracts of N sativa seeds have anti-inflammatory and antioxidant activities, and being used by patients to suppress coughs, disintegrate renal calculi, retard the carcinogenic process, treat abdominal pain, diarrhea, flatulence and polio (Ahmad et al., 2013; Al-Khalaf and Ramadan, 2013). The seed of this plant, commonly known as black seed, are eaten alone or in combination with honey and in many food preparations and the oil prepared by compressing the seeds of N sativa is used for cooking (Al-Khalaf and Ramadan, 2013). The seeds of N sativa contain both fixed and essential oils, proteins, alkaloids and saponin (Ali and Blunden, 2003; Khan et al., 2011). Many active ingredients found in the seeds of N sativa have beneficial effects against various cancer diseases, including cervical cancer (Effenberger et al., 2010), blood cancer (El-Mahdy et al., 2005), hepatic cancer (Thabrew et al., 2005), colon cancer (Salim and Fukushima, 2003), pancreatic cancer (Chehl et al., 2009), skin cancer (Salomi et al., 1991), fibrosarcoma (Awad, 2005), renal cancer (Khan and Sultana, 2005), prostate cancer (Yi et al., 2008), and breast cancers (Farah and Begum, 2003; Ahmad et al., 2012). Pharmacologically important components of N sativa extracts have also been studied against lung cancer as an anticancer agent. In one of the study Swamy and Huat (2003) have shown the antitumor activity of α-hederin from N sativa against Lewis lung carcinoma in BDF1 mice. Protective effect of N sativa extracts against methyl nitrosourea-induced oxidative stress, inflammatory response and carcinogenesis in lung cells has also been shown (Mabrouk et al., 2002). These studies showed that N sativa extracts can protect lung cells, but the molecular mechanisms of N sativa extracts against lung cancer cells have not been explored till date. Therefore, the present study was designed to investigate the in vitro cytotoxic activity of N sativa seed extracts against human lung cancer cell line A-549.

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Interaction of Al₂O₃ nanoparticles with Escherichia coli and their cell envelope biomolecules

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Keywords
Al₂O₃ NPs, AT-FTIR, ESBL, HR-TEM, LPS, PE.

Abstract

Aims: The aim of this study is to investigate the antibacterial activity of aluminium oxide nanoparticles (Al₂O₃ NPs) against multidrug-resistant clinical isolates of Escherichia coli and their interaction with cell envelope biomolecules.

Methods and Results: Al₂O₃ NPs were characterized by scanning electron microscope (SEM), high-resolution transmission electron microscope (HR-TEM) and X-ray diffraction (XRD) analyses. Antibacterial activity and interaction of Al₂O₃ NPs with E. coli and its surface biomolecules were assessed by spectrophotometry, SEM, HR-TEM and attenuated total reflectance/Fourier transform infrared (ATR-FTIR). Of the 80 isolates tested, about 64 (80%) were found to be extended spectrum β-lactamase (ESBL) positive and 16 (20%) were non-ESBL producers. Al₂O₃ NPs at 1000 μg ml⁻¹ significantly inhibited the bacterial growth. SEM and HR-TEM analyses revealed the attachment of NPs to the surface of cell membrane and also their presence inside the cells due to formation of irregular-shaped pits and perforation on the surfaces of bacterial cells. The intracellular Al₂O₃ NPs might have interacted with cellular biomolecules and caused adverse effects eventually triggering the cell death. ATR-FTIR studies suggested the interaction of lipopolysaccharide (LPS) and L-α-Phosphatidyl-ethanolamine (PE) with Al₂O₃ NPs. Infrared (IR) spectral changes revealed that the LPS could bind to Al₂O₃ NPs through hydrogen binding and ligand exchange. The Al₂O₃ NPs-induced structural changes in phospholipids may lead to the loss of amphiphilic properties, destruction of the membrane and cell leaking.

Conclusions: The penetration and accumulation of NPs inside the bacterial cell cause pit formation, perforation and disorganization and thus drastically disturb its proper function. The cell surface biomolecular changes revealed by ATR-FTIR spectra provide a better understanding of the cytotoxicity of Al₂O₃ NPs.

Significance and Impact of the Study: Al₂O₃ NPs may serve as broad-spectrum bactericidal agents to control the emergent pathogens regardless of their drug-resistance mechanisms.

Introduction

The re-emergence of infectious diseases and the continuous development of antibiotic resistance among a variety of disease-causing bacteria pose a serious threat to public health worldwide (Adams et al. 2006). Due to extensive use of β-lactam antibiotics over the last several decades in the clinical practice, various new classes of β-lactamases
Hepatoprotective potential of *Lavandula coronopifolia* extracts against ethanol induced oxidative stress-mediated cytotoxicity in HepG2 cells

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Abstract

The present investigations were carried out to study the protective potential of four extracts (namely petroleum ether extract (LCR), chloroform extract (LCM), ethyl acetate extract (LCE), and alcoholic extract (LCL)) of *Lavandula coronopifolia* on oxidative stress-mediated cell death induced by ethanol, a known hepatotoxin in human hepatocellular carcinoma (HepG2) cells. Cells were pretreated with LCR, LCM, LCE, and LCL extracts (10–50 μg/ml) of *L. coronopifolia* for 24 h and then ethanol was added and incubated further for 24 h. After the exposure, cell viability using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and neutral red uptake assays and morphological changes in HepG2 cells were studied. Pretreatment with various extracts of *L. coronopifolia* was found to be significantly effective in countering the cytotoxic responses of ethanol. Antioxidant properties of these *L. coronopifolia* extracts against reactive oxygen species (ROS) generation, lipid peroxidation (LPO), and glutathione (GSH) levels induced by ethanol were investigated. Results show that pretreatment with these extracts for 24 h significantly inhibited ROS generation and LPO induced and increased the GSH levels reduced by ethanol. The data from the study suggests that LCR, LCM, LCE, and LCL extracts of *L. coronopifolia* showed hepatoprotective activity against ethanol-induced damage in HepG2 cells. However, a comparative study revealed that the LCE extract was found to be the most effective and LCL the least effective. The hepatoprotective effects observed in the study could be associated with the antioxidant properties of these extracts of *L. coronopifolia*.

Keywords

*L. coronopifolia* extracts, HepG2 cells, ethanol, cytotoxicity, oxidative stress

Introduction

Liver diseases are the major public health problems worldwide (Asha and Pushpangadan, 1998), since liver plays a key role in the regulation of various physiological processes in the human body. A variety of chemicals like alcohol, carbon tetrachloride, tert-butyl hydroperoxide, acetaminophen, and paracetamol can cause potential damage to the liver cells leading to progressive liver dysfunction (Joyeux et al., 1990; Nithianantham et al., 2011; Rodeiro et al., 2008). The role of oxidative stress and lipid peroxidation (LPO) in liver cells induced by various hepatotoxins are well known (Rodeiro et al., 2008). Evidences from various studies show that overproduction...
Antibacterial properties of silver nanoparticles synthesized using *Pulicaria glutinosa* plant extract as a green bioreductant

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Abstract: The antibacterial properties of nanoparticles (NPs) can be significantly enhanced by increasing the wettability or solubility of NPs in aqueous medium. In this study, we investigated the effects of the stabilizing agent on the solubility of silver NPs and its subsequent effect on their antimicrobial activities. Silver NPs were prepared using an aqueous solution of *Pulicaria glutinosa* plant extract as bioreductant. The solution also acts as a capping ligand. During this study, the antimicrobial activities of silver NPs, as well as the plant extract alone, were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Micrococcus luteus*. Silver NPs were prepared with various concentrations of the plant extract to study its effect on antimicrobial activity. Interestingly, various concentrations of *P. glutinosa* extract did not show any effect on the growth of tested bacteria; however, a significant effect on the antimicrobial property of plant extract capped silver NPs (Ag-NPs-PE) was observed. For instance, the half maximal inhibitory concentration values were found to decrease (from 4% to 21%) with the increasing concentrations of plant extract used for the synthesis of Ag-NPs-PE. These results clearly indicate that the addition of *P. glutinosa* extracts enhances the solubility of Ag-NPs-PE and, hence, increases their toxicity against the tested microorganisms.

Keywords: antibacterial activity, silver nanoparticles, plant extract, *Pulicaria glutinosa*

Introduction

Among the greatest health challenges in recent times, and one of the most serious concerns, is the emergence of antibiotic-resistant bacteria that have developed resistance against many conventional antibacterial agents.¹⁻⁴ This has prompted the advancement of alternative strategies, including the application of nanoparticles (NPs) as antimicrobial agents.⁵ Because of their high surface-to-volume ratio and unique physical and chemical properties, which are different from their bulk properties, NPs have demonstrated excellent antimicrobial activity.⁶⁻⁹ Several types of nanomaterials and their composites, including copper, zinc, titanium, gold, and silver, have been applied as antimicrobial agents.¹⁰⁻¹³ Despite some genuine concern regarding their toxicity,¹⁴ silver NPs exhibit excellent antimicrobial activity against bacteria, viruses, and other eukaryotic microorganisms.¹⁵⁻¹⁸

Numerous studies have attempted to explain the mechanisms by which silver NPs exert their antibacterial activities.¹⁹⁻²¹ In general, the surface area of the NPs and their ability to effectively release silver ions is the key to their antibacterial activity. The extremely large surface area of NPs facilitates better contact with microorganisms, because of which NPs get easily attached to the cell membrane and also penetrate inside the bacteria.²² The release of ionic silver inactivates vital bacterial enzymes, inhibits...
Anti-biofilm and antibacterial activities of zinc oxide nanoparticles against the oral opportunistic pathogens Rothia dentocariosa and Rothia mucilaginosa


Species of the genus Rothia that inhabit the oral cavity have recently been implicated in a number of diseases. To minimize their role in oral infections, it is imperative to reduce and/or control the growth and biofilm formation activity of Rothia spp. In this study, two bacterial isolates, Ora-7 and Ora-16, were obtained from the oral cavity of a healthy male subject and identified as Rothia dentocariosa and Rothia mucilaginosa, respectively, using a polyphasic taxonomic approach. Antimicrobial and anti-biofilm formation activities of zinc oxide nanoparticles (ZnO-NPs), of average size 35 nm, were assessed in in vitro assays using Crystal Violet and live and dead staining techniques. The ZnO-NPs exhibited an IC₅₀ value of 53 and 76 µg ml⁻¹ against R. dentocariosa and R. mucilaginosa, respectively. Biofilm formation assays, performed on the surfaces of polystyrene plates, artificial teeth, and dental prostheses, revealed the efficacy of ZnO-NPs as a potential antibacterial agent for controlling the growth of Rothia isolates in both planktonic form and biofilm.

The genus Rothia consists of six species, three of which, viz. Rothia dentocariosa, Rothia mucilaginosa, and Rothia aeria, are common inhabitants of the oral cavity and are essentially benign. Recent reports suggest these species to be opportunistic pathogens, causing a number of diseases in addition to dental and periodontal ailments (1). R. dentocariosa, originally isolated from carious lesions of human teeth, has been found to cause endocarditis (2), pneumonia (3), and infections of the peritoneum and lung (4). Similarly, R. mucilaginosa isolated from mouse (5) has been reported to cause bacteraemia (6) and pneumonia (7). Chavan et al. (8) have shown that infection with R. mucilaginosa in children with haematological malignancies or following haematopoietic stem cell transplantation results in a higher rate of mortality, and R. aeria (isolated from the air of a Russian space laboratory) has been reported as the causative agent of acute bronchitis (9) and sepsis in neonates (10). Therefore, these early studies have suggested that Rothia spp. are infective opportunistic pathogens, especially in immunocompromised patients (3, 11, 12). Because Rothia infections pose a much more serious health issue than previously thought, the development of simple and effective approaches to minimize and/or prevent their infectivity is crucial.

Growing resistance of bacteria to traditional antibiotics is a critical problem, even amongst oral bacteria (13), which is further complicated by the formation of biofilm on oral surfaces by these bacteria owing to the fact that biofilms exhibit significantly greater resistance to antibiotics than do planktonic cells, resulting in the development of chronic and recurring infections. Recently, metal oxide nanoparticles (NPs) have been regarded as promising alternatives to traditional antimicrobial agents (14–17). A number of reports have demonstrated the antimicrobial and antibiofilm activities of NPs against pathogenic bacteria (16, 17). Zinc oxide NPs (ZnO-NPs) have been reported to exhibit remarkable antimicrobial and anti-biofilm activity against oral bacteria (14), and are known to inhibit dentine demineralization (18). In this study, we investigated the antimicrobial and anti-biofilm potential of ZnO-NPs against the oral bacterial isolates R. dentocariosa (Ora-7) and R. mucilaginosa (Ora-16).
Concentration-Dependent Induction of Reactive Oxygen Species, Cell Cycle Arrest and Apoptosis in Human Liver Cells After Nickel Nanoparticles Exposure

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ABSTRACT:: Due to advent of nanotechnology, nickel nanoparticles (Ni NPs) are increasingly recognized for their utility in various applications including catalysts, sensors and electronics. However, the environmental and human health effects of Ni NPs have not been fully investigated. In this study, we examined toxic effects of Ni NPs in human liver (HepG2) cells. Ni NPs were prepared and characterized by X-ray diffraction, transmission electron microscopy and dynamic light scattering. We observed that Ni NPs (size, ~28 nm; concentration range, 25–100 μg/mL) induced cytotoxicity in HepG2 cells and degree of induction was concentration-dependent. Ni NPs were also found to induce oxidative stress in dose-dependent manner evident by induction of reactive oxygen species and depletion of glutathione. Cell cycle analysis of cells treated with Ni NPs exhibited significant increase of apoptotic cell population in subG1 phase. Ni NPs also induced caspase-3 enzyme activity and apoptotic DNA fragmentation. Upregulation of cell cycle checkpoint gene p53 and bax/bcl-2 ratio with a concomitant loss in mitochondrial membrane potential suggested that Ni NPs induced apoptosis in HepG2 cells was mediated through mitochondrial pathway. This study warrants that applications of Ni NPs should be carefully assessed as to their toxicity to human health.

Keywords: nickel nanoparticles; human liver cells; health effects; oxidative stress; cell cycle

INTRODUCTION

Metallic nickel (Ni) and Ni compounds are released into the atmosphere during mining, smelting, and refining operations representing an environmental and industrial pollutant (Cavallo et al., 2003; Magaye and Zhao, 2012). Evidence
Punicalagin and Ellagic Acid Demonstrate Antimutagenic Activity and Inhibition of Benzo[a]pyrene Induced DNA Adducts

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Punicalagin (PC) is an ellagitannin found in the fruit peel of Punica granatum. We have demonstrated antioxidant and antigenotoxic properties of Punica granatum and showed that PC and ellagic acid (EA) are its major constituents. In this study, we demonstrate the antimutagenic potential, inhibition of BP-induced DNA damage, and antiproliferative activity of PC and EA. Incubation of BP with rat liver microsomes, appropriate cofactors, and DNA in the presence of vehicle or PC and EA showed significant inhibition of the resultant DNA adducts, with essentially complete inhibition (97%) at 40 μM by PC and 77% inhibition by EA. Antimutagenicity was tested by Ames test. PC and EA dose-dependently and markedly antagonized the effect of tested mutagens, sodium azide, methyl methanesulfonate, benzo[a]pyrene, and 2-aminofluorine, with maximum inhibition of mutagenicity up to 90 percent. Almost all the doses tested (50–500 μM) exhibited significant antimutagenicity. A profound antiproliferative effect on human lung cancer cells was also shown with PC and EA. Together, our data show that PC and EA are pomegranate bioactives responsible for inhibition of BP-induced DNA adducts and strong antimutagenic, antiproliferative activities. However, these compounds are to be evaluated in suitable animal model to assess their therapeutic efficacy against cancer.

1. Introduction

Over the past few decades, tremendous outcomes have been resulted by exploring antioxidant and antimutagenic potential of medicinal plants. It is widely accepted that oxidative modification of DNA, protein, lipids, and small cellular molecules by both exogenous and endogenous reactive oxygen species including free radicals and nonfree radicals plays an important role in a wide range of common diseases including cancer and age related degenerative diseases [1, 2]. The human body possesses innate defence mechanisms to counter free radicals. Plant secondary metabolites such as phenolics, flavonoids, and terpenoids play an important role in the defence against free radicals [3]. Moreover, these natural antioxidants may reduce or inhibit the mutagenic potential of mutagens, promutagens, and carcinogens [4, 5]. Therefore, the discovery and the exploration of compounds possessing antioxidant, antimutagenic, and anticancer properties are now fetching great practical and therapeutic significance.

The formation of DNA adducts (i.e., carcinogens covalently bound to DNA) is widely considered a prerequisite for the initiation and progression of cancer development. Many carcinogens are known to induce the formation of DNA adducts [6] and the presence of DNA adducts in humans has been strongly correlated with an increased risk of cancer development [7]. For example, human studies have shown a higher accumulation of tissue DNA adducts in cigarette smokers than in nonsmokers or individuals who have never smoked, indicating that DNA adduct formation is a viable target for the treatment of cancer [8].

Benzo[a]pyrene (BP) is one of the most potent and extensively studied carcinogens. In a cellular system, BP is metabolized to the electrophilic metabolite, benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), that attaches covalently to DNA.
In vitro Assessment of Antioxidant Activity of Selected Essential Oils
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Abstract- Essential oils are the liquid mixture of volatile compounds obtained by steam distillation from various plants. Many essential oils are known for biological properties. Considering the growing demand of natural antioxidants, three essential oils were tested for their antioxidant activity using DPPH radical scavenging assay and Ferric reducing antioxidant power (FRAP). These oils were also assayed for their total phenolic content. Free radical scavenging activity was found maximum in Thyme oil followed by Eucalyptus oil and Peppermint oil. However in FRAP method, the order of antioxidant power was maximum for Thyme oil followed by Peppermint oil > Eucalyptus oil. A concentration dependent increase in antioxidant activity was also recorded in both assays. Further HPTLC and GC-MS based detection of active compound is needed to understand the mechanism and synergy among various compounds.

Keywords- Essential oil, Antioxidant activity, Polyphenolics, Free radical scavenging.

Introduction
Medicinal plants and their derived products are important therapeutic utility for number of ailments; it has been recorded since ancient times that different parts of medicinal plants are used to cure specific ailments. Owing to wide acceptance for the safety and reliability of these natural entities as compared to expensive and possible adversarial effects comprising synthetic drugs, there is widespread interest in drug derived from plants (Gordon et al., 2001). The custom of indigenous plants in traditional health practices has a long past. Medicinal plants are used to preserve and promote healthy life, prevent disease and cure ailments. It has been assessed that even today, 80% of the world population rely on herbal traditional medicines for their primary health care (Cassady et al., 1990; Cragg et al., 2005; Ahmad et al., 2006).

Aromatic and medicinal plants are the source of natural antioxidants due to their secondary metabolites such as polyphenols. Phenolics can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation (Lapornik et al., 2005). Essential oils also called volatile or ethereal oils are aromatic oily liquids obtained from different plant parts and widely used as food flavours (Burt, 2004). The use of essential oils has been well established in food preservation, pharmaceuticals, alternative medicines and natural therapies (Bruckdorfer, 1996; Lis-Balchin and Deans, 1997). Essential oils are complex mixture of terpenes and their compounds. Because of their relatively safe status, essential oils and their constituents are gaining increasing interest, and their exploitation for potential multi-purpose functional use (Ormancey et al., 2001; Sawamura, 2000).

In the recent past, essential oils are reported for their various biological properties such as antiseptic, antifungal, antibiofilm, anti-Quorum Sensing, anti-inflammatory as well antioxidant activities (Ahmad et al., 1998; Ahmad et al., 1999; Beg and Ahmad 2002; Khan and Ahmad 2012; Hussein et al., 2013; Amorati et al., 2014). Further, there is an increase demand for safe antioxidant agents to be used in cosmetics, pharmaceuticals, food preservations and healthcare (Amorati et al., 2014). Though the chemical antioxidants (BHA, BHT etc.) have shown to be toxic in nature (Sasaki et al., 2002), yet the role of essential oil in traditional system of medicine, especially in aromatherapy is well established (Buttner et al., 1996). The search for natural antioxidants has generated interest among scientific community to reinvestigate essential oils as safe and promising antioxidant agent (Wannes et al., 2010; Tongpoothorn et al., 2012; Kapoor et al., 2014). Some reports on essential oil property are contradictory also. Therefore, using more than one method for assessing antioxidant property of essential oil will provide comparative analysis which may be useful in se-
ANTIBIOTIC RESISTANCE PROFILE OF L. MONOCYTOGENES ISOLATED FROM FOOD SOURCES

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ABSTRACT

The infection caused by L. monocytogenes is commonly known as listeriosis. The disease occurs in humans and a variety of animals. The bacterium L. monocytogenes has considered as an important foodborne pathogen in all over the world. The occurrence of antibiotic resistance among L. monocytogenes is posing problem in treatment of the disease. In this study, the in vitro testing of antibiotic susceptibility among twenty four food isolates of L. monocytogenes showed highest (33.3%) resistance to penicillin. Further a slightly lower (20.8%) resistance to chloramphenicol and tetracycline each was observed. To the ampicillin, ciprofloxacin, cephalothin, gentamycin and trimethoprim a lower range of resistance from 4.1-12.5% was observed. In this study, ciprofloxacin and gentamycin were observed as most effective antibiotic against tested isolates to which only 4.1% and 8.3% resistance were observed respectively. Apart from this, the multiple drug resistant among 3 (8.3%) L. monocytogenes isolates was also observed. The presence of L. monocytogenes in foodstuffs is a serious problem. This study indicates significant resistance among isolates of L. monocytogenes from food sources. That can pose the problem in treatment of L. monocytogenes infections as resistant strains may also transfer the resistance to other microorganisms. Therefore, the multiple drug resistance among L. monocytogenes is alarming an increase potential threat to human health posed by this pathogen through the consumption of contaminated meat, milk and milk products in Bareilly city, India.

KeyWords: Food; L. monocytogenes; Antibiotic resistance; multiple drug resistance.

INTRODUCTION

Listeria monocytogenes is a gram positive, facultative anaerobic, non-acid fast, and a non-spore forming rod that expresses a typical tumbling motility at 20-25°C. The bacterium is a highly adaptable food-borne pathogen that causes the life threatening illness listeriosis in infected individuals (Kaur et al. 2007; Adzitey and Huda 2010). The organism has been isolated from various animal food products such as meat, milk and their products associated with many listeriosis outbreaks. Therefore, contaminated foods are considered a primary source of transmission of infection in sporadic cases as well as outbreaks (Dumen et al. 2008; Latorre et al. 2009; Jami et al. 2010).

The monitoring of foodborne pathogens presence in different type of foods is primary...
Antimicrobial Efficacy and Interaction of Plant Extracts With and Without Antibiotics Against Drug Resistant Bacteria

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Abstract: New sources of antimicrobial drugs need to be identified and improved strategy should be developed to combat multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating antimicrobial action needs to be exploited for their synergistic action between extracts and with antibiotics to exploit it in modern phytomedicine and combinational therapy. In the present study, alcoholic extracts of fifteen medicinal plants were screened for their antimicrobial efficacy against a wide variety of drug resistant bacteria and yeast. The extracts of Carum copticum, C. juncea, H. spicatum, Z. officinale, S. aromaticum, Camellia sinensis, T. foenum graecum, Piper cubeba, C. longa and A. barbadensis showed promising action against one or more drug resistant bacteria as well as against Candida albicans with MIC ranged from 0.5 mg/ml to 9.5 mg/ml. Many combinations of these extracts showed synergistic action. The extract of Carum copticum exhibited synergy with antibiotics, tetracycline, chloramphenicol, ampicillin and gentamicin against methicillin resistant S. aureus which has indicated their potential to be exploited in combination drug therapy after careful evaluation in vivo model.

Key words: Antimicrobial activity, MDR bacteria, MIC, synergistic activity antibiotics

Introduction
The use of herbal and other natural substances is part of the fabric of traditional medicine in different part of the world. Medicinal plants have been found good source of therapeutic and novel compounds. Targeted screening of a large diversity of medicinal plants is expected to yield novel biological activities including problematic group of multidrug resistant bacterial pathogens (Ahmad et al., 2008).

Bacteria have evolved numerous defenses against antimicrobial agents and drug resistant pathogens are on the rise and such bacteria have become a global health problem. Nearly twenty years ago over 90% S. aureus strains were reported b-lactamase positive. Strains of b-lactam resistant Staphylococcus aureus including MRSA now pose a serious problem to hospitalized patients and their care providers (Liu, et al., 2000). The production of b-lactamase is recognized as one of the main mechanism of bacterial-resistance to b-lactamase antibiotics. Numerous compound have been included in the list of b-lactamase inhibitors and some of these have shown potential clinical usefulness based on their synergistic-effects when they are combined with b-lactamase-labile antibiotics. Many b-lactamase were found to be resistant to b-lactamase inhibitors. Similarly multidrug resistant problem is common in members of family Enterobacteriaceae specially E.coli, Salmonella, Shigella and several other humans and animal pathogen like Haemophilus influenzae, Campylobacter, Pseudomonas aeruginosa, Mycobacterium tuberculosis both in developing and developed countries (Eldelstein et al., 2001; Tonkic et al., 2005; Ahmad et al., 2008).

India has one of the world’s richest flora with about 120 families of plant comprising 1, 30,000 species. A large portion of the world population especially in the developing countries depends on the traditional system of medicine for a variety of diseases. The world health organization (WHO) reported that 80% of the world’s population rely chiefly on traditional medicines and major part of the traditional therapies in-
Carum copticum and Thymus vulgaris oils inhibit virulence in Trichophyton rubrum and Aspergillus spp

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Abstract

Emergence of drug-resistant strains has demanded for alternative means of combating fungal infections. Oils of Carum copticum and Thymus vulgaris have long been used in ethnomedicine for ailments of various fungal infections. Since their activity has not been reported in particular against drug-resistant fungi, this study was aimed to evaluate the effects of oils of C. copticum and T. vulgaris on the growth and virulence of drug-resistant strains of Aspergillus spp. and Trichophyton rubrum. The gas chromatography-mass spectrometry analysis revealed thymol constituting 44.71% and 22.82% of T. vulgaris and C. copticum, respectively. Inhibition of mycelial growth by essential oils was recorded in the order of thymol > T. vulgaris > C. copticum against the tested strains. RBC lysis assay showed no tested oils to be toxic even up to concentration two folds higher than their respective MFCs. Thymol exhibited highest synergy in combination with fluconazole against Aspergillus fumigatus MTCC2550 (FICI value 0.187) and T. rubrum IOA9 (0.156) as determined by checkerboard method. Thymol and T. vulgaris essential oil were equally effective against both the macro and arthroconidia growth (MIC 72/109 g/mL). A > 80% reduction in elastase activity was recorded for A. fumigatus MTCC2550 by C. copticum, T. vulgaris oils and thymol. The effectiveness of these oils against arthroconidia and synergistic interaction of thymol and T. vulgaris with fluconazole can be exploited to potentiate the antifungal effects of fluconazole against drug-resistant strains of T. rubrum and Aspergillus spp.

Key words: anti-elastase activity, arthroconidia, synergy, thymol, virulence.

Introduction

Fungal infections caused by various pathogenic and opportunistic strains are on the rise in the different parts of the world. This is primarily due to growing number of high risk patients particularly immunocompromised. Filamentous fungi including Aspergillus fumigatus and dermatophytes are common reported pathogens (Vermount et al., 2008; Dagenais and Keller, 2009). Invasive aspergillosis caused by Aspergillus spp. in immunocompromised hosts may cause morbidity and mortality in a range from 40 to 90% in high risk populations (Dagenais and Keller, 2009). Whereas, increased incidence of dermatophytooses, infections of hair, skin and nails, caused by Trichophyton spp., have been reported in recent years especially in the tropical countries (Vazquez, 2003). Such infections are not life-threatening; however, both immunocompetent and immunosuppressed persons are affected. Such infections have increased considerably among pediatric and geriatric populations (Monod, 2008) and can become serious in immunocompromised patients resulting in invasive infections (Sokovic et al., 2008). Hence, the management of these fungal infections would be a definite challenge to mankind.

In spite of introduction of newer antifungal drugs, such fungal infections have been threatened by the development of drug resistant strains, host toxicity and variable drug bioavailability (Barker and Rogers, 2006). Also, these drugs are costly and not affordable to a larger section of human population across the globe (Barker and Rogers, 2006; Baddley and Pappas, 2007; Ahmad et al., 2010). Therefore,
Synthesis and antimicrobial evaluation of fatty chain substituted 2,5-dimethyl pyrrole and 1,3-benzoxazin-4-one derivatives

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KEYWORDS
Condensation; Cyclization; Acetonyl acetone; Anthranilic acid; Structure activity relationship

Abstract Fatty acids themselves have a number of biological properties and its easy intake by the human body will focus to the synthesis of many heterocyclic moiety substituted with fatty acid residue, to make more gradual intake of heterocycles in the human body. 2,5-Dimethyl pyrrole 2(a-e) and 1,3-benzoxazin-4-one 4(b-e) derivatives were synthesized, from cyclization of fatty acid hydrazide 1(a-e) with acetonyl acetone and from the reaction of fatty esters 3(b-e) with anthranilic acid in the presence of POCl3, respectively. All these compounds were characterized with the help of IR, 1H NMR, 13C NMR and mass spectra. The synthesized compounds were screened for antimicrobial evaluation against gram-positive (Staphylococcus aureus SA 22, Bacillus subtilis MTCC 121), gram-negative (Escherichia coli K12, Klebsiella pneumoniae) and fungal strains (Candida albicans IOA-109) and were found to be good antimicrobial agents.

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1. Introduction

The pyrrole ring is a part of many biological compounds such as the enzyme catalase, the bile pigment bilirubin and the mould pigment prodigiosin; it is also a significant part of macrocyclic porphyrin ring system of chlorophyll and hemin [1,2]. Apart from these properties pyrrole and its derivative possess a number of biological activities such as antiallergic, antitumor [3], antibacterial, antifungal [4], antiinflammatory, analgesic [5], anticonvulsant [6], antimycobacterial [7] antitubercular, anticancer [8] and anti HIV [9]. Substituted dimethyl pyrroles can be synthesized from the widely used Knorr pyrrole synthesis [10]. Other methods are also known for the synthesis of 2,5-dimethyl pyrrole derivatives [11,12]. Sometimes for the synthesis of substituted pyroles, photochemical reactions are also used, which involves the use of other pyrrole precursor including the migration of group from one nitrogen atom to the ring carbon atom [13]. Despite the biological use of substituted dimethyl pyrroles they have been synthetically...
Flower-shaped ZnO nanoparticles synthesized by a novel approach at near-room temperatures with antibacterial and antifungal properties

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Abstract: Due to enormous applications of metal oxide nanoparticles in research and health-related applications, metal oxide nanoparticles are increasingly being developed through cheaper and more user-friendly approaches. We have formulated a simple route to synthesize zinc oxide nanoparticles (ZNPs) by a sol–gel method at near-room temperatures 25°C, 35°C, 55°C, and 75°C. The results are analyzed by X-ray diffraction, scanning electron microscopy with energy-dispersive X-ray spectroscopy, and ultraviolet-visible absorption spectroscopy. The effect of different temperature conditions (25°C–75°C) on the particulate sizes (23.7–88.8 nm), pH levels (11.7–11.9), and morphologies (slender needle–broad arrow) of flower-shaped ZNP colonies is studied. A possible mechanism depicting the growth rates at different temperatures and of different facets, mainly towards the <0001> and <01̅1̅0> planes of the ZNPs has also been discussed. The values of λmax (293–298 nm) suggest that ZNPs prepared at 55°C are the most effective ultraviolet B absorbers, and that they can be used in sunscreens. Highly significant antimicrobial activity against medically important Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria and fungi (Candida albicans) by these ZNPs was also revealed. As S. aureus and C. albicans are responsible for many contagious dermal infections such as abscesses, furuncles, carbuncles, cellulitis, and candidiasis, we can postulate that our fabricated ZNPs may be useful as antimicrobial agents in antiseptic creams and lotions for the treatment of skin diseases.

Keywords: antimicrobial activity, cetyl trimethyl ammonium bromide, flower-shape zinc oxide nanoparticles, near-room temperature, sol–gel method, skin disease

Introduction

Zinc oxide (ZnO) is an n-type semiconductor that has a wide band gap of approximately 3.3 eV, along with a large excitation binding energy of 60 meV (at 298 K).1–2 ZnO has the good property of being able to produce blue-green luminescence and absorption in the ultraviolet (UV) region, which is exploited for sunscreens, textile industries, catalysts, sensors, photodetectors, and for obtaining solar energy.3–10 Keeping the scope of its applications in mind, the different morphologies of ZnO nanoparticles (ZNPs) are developed as nanoflowers, nanorods, nanowhiskers, nanobelts, nanotubes, nanorings, nanocolumns, and so on.11–17

Different methods that are used for the production of ZNPs include the sol–gel method, facile hydrothermal method, solution method, electric current heating method; solvothermal method, self-propagating high-temperature synthesis method, spontaneous nucleation method, spray pyrolysis, gas-phase reaction method, laser ablation method, thermal evaporation, and so on.18–31 The different parameters that are generally used require temperatures ranging from low to more than 1,500°C, pressures from 1 atm to a...
Heterobimetallic o-vanillin functionalized complexes: In vitro DNA binding validation, cleavage activity and molecular docking studies of Cu$^{II}$–Sn$^{IV}$ analogs

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**Abstract**

The heterobimetallic chemical entities 1–4 of o-vanillin functionalized Schiff base have been synthesized and characterized by elemental analysis and spectroscopic methods viz., UV–vis, IR, ESI–mass, NMR (in 2 and 4) and EPR (in 1 and 3). The Ni$^{II}$–Sn$^{IV}$ analogs were synthesized only for structural elucidation by NMR spectroscopy. To evaluate the biological preference with the molecular target DNA, interaction of the Cu$^{II}$–Sn$^{IV}$ entities 1 and 3 with CT DNA has been explored by employing various biophysical methods revealing the electrostatic mode of binding via oxygen of sugar–phosphate backbone of DNA helix. The $k_b$ values of 1 and 3 were found to be $2.31 \times 10^4$ and $3.67 \times 10^3$ M$^{-1}$, respectively suggesting the greater binding propensity of 3. Furthermore, site of action was ascertained by the interaction studies of 1 and 3 with 5’-AMP employing UV–vis titrations. $^{1}$H and $^{31}$P NMR studies which implicates the preferential selectivity of these complexes to N1 of adenosine moiety. Moreover, the antimicrobial activities of 1 and 3, revealed 3 as a good antimicrobial agent. The cleavage activity of 3 was evaluated by agarose gel electrophoresis assay with pBR322 DNA, revealing the involvement of singlet oxygen species via oxidative cleavage pathway. Additionally, 3 exhibited significant inhibitory effects on the catalytic activity of Topo I at a very low concentration, 15 μM, suggesting that 3 is an efficient catalytic inhibitor of human Topo I. The computer-aided molecular docking techniques were carried out to ascertain the mode of action toward the molecular target DNA and Topo I for 1 and 3.

**Keywords:** Cu$^{II}$–Sn$^{IV}$ complex, In vitro DNA profiling, 5’-AMP, Oxidative cleavage, Topo I inhibition, Molecular docking

1. Introduction

Cancer is leading cause of mortality globally, accounting for 7.6 million deaths around the world in 2008, and an estimated 13.1 million deaths by 2030 [1]. The serendipitous discovery of cisplatin, cis-diamminedichloroplatinum (II) – an archetypical inorganic anticancer drug [2], has triggered the design of new improved metal-based chemotherapeutic agents with fewer side effects. The role of cisplatin, its second generation analogs viz., carboplatin, oxaliplatin, etc. and multinuclear complexes like BBR3464 as chemotherapeutic anticancer drugs have been well established [3]. However, the clinical effectiveness of the existing anticancer drugs was not good enough owing to severe side effects [4] and acquisition of resistance by tumor cells [5]. To address these limitations, optimization of the chemical entities to exert effective chemotherapeutic potential was needed.

The chemotherapeutic anticancer drugs exert their cytotoxic effect, and thereby therapeutic effect by interacting with DNA, topoisomerases or DNA–topoisomerase complexes. DNA Topo I is a ubiquitous cellular enzyme that catalyzes the topological changes of DNA during fundamental cellular processes such as replication, transcription, recombination and repair by triggering single-stranded breaks in DNA [6]. Topoisomerase targeting compounds are considered as an attractive target for design of cancer chemotherapeutics, because they can cause permanent DNA damage that triggers a series of cellular events, inducing apoptosis leading to cell death [7]. In this respect, DNA Topo I inhibitors represent a class of anticancer agents forming the basis of many chemotherapy combinations widely used in a broad spectrum of tumors. A series of drugs that specifically target DNA Topo I, such as camptothecin (CPT) and its derivatives, as well as other non-CPT Topo I inhibitors like indenoisoquinolines have been used clinically as antitumor agents in cancer chemotherapy [8].

**Abbreviations:** CT DNA, calf thymus DNA; ER, ethidium bromide; En, ethylendiamine; Topo I, topoisomerase I; UV–vis, UV–visible.

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Enantiomeric in vitro DNA binding, pBR322 DNA cleavage and molecular docking studies of chiral L- and D-ternary copper(II) complexes of histidine and picolinic acid

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Novel chiral ternary Cu(II) and Ni(II) complexes of L/D-histidine and picolinic acid, 1 and 2(a and b) were synthesized and characterized by elemental analysis, molar conductance and spectroscopic data (IR, NMR, EPR, UV–vis). In vitro DNA binding profile of both Cu(II) and Ni(II) complexes have been investigated by UV–vis titrations, while fluorescence spectroscopy, circular dichroism and viscosity measurements were carried out for Cu(II) complexes 1(a and b). Both the enantiomers of 1 and 2(a and b) bind to CT DNA via electrostatic interactions and the intrinsic binding constant, K0 values for complexes 1 and 2(a and b) were found to be 5.6 × 104, 9.8 × 104, 8.2 × 104 and 6.7 × 103 M−1, respectively suggesting greater binding propensity of L-form of Cu(II) complex 1a. The DNA cleavage activity of complexes 1(a and b), investigated by agarose gel electrophoresis suggested an oxidative pathway for DNA cleavage. Further, the molecular docking studies of complexes 1(a and b) were carried out with B-DNA revealing that the complexes bind to the adenine–thymine residues in the minor groove of the DNA. The resulting binding energies of docked metal complexes 1(a and b) were found to be −265.1 and −218.9 KJ mol−1, respectively. Furthermore, enantiomeric complexes 1 and 2(a and b) were screened for in vitro antimicrobial activity.

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1. Introduction

There is a growing interest in developing agents that can target specific DNA sequences, for application in chemotherapy [1], in the development of tools for biotechnology [2], and for nucleic acid structural probes. These molecules can be exploited as chemotherapeutic agents for treating a variety of diseases viz., AIDS–HIV, cancer etc. Most of the chemotherapeutic anticancer drugs exert their cytotoxic effect and thereby therapeutic effect by interacting with DNA. After the serendipitous discovery of clinically approved chemotherapy drug-cisplatin, transition metal complexes have been utilized for the design and development of robust therapeutic drugs that bind to nucleic acid. Metal complexes can interact with DNA duplex through a variety of binding modes, DNA intercalation [3], covalent binding [4] and non-covalent [5,6] (electrostatic, groove binding and hydrogen bonding) interactions. Metal complexes have great diversity in size and structure, as well as possess useful spectroscopic, photo-physical and electrochemical properties, and consequently they can become an important class of structure-selective binding agents for nucleic acids.

DNA is an inherently chiral molecule; the asymmetric D-ribose and D-deoxyribose units contain several stereogenic centers, whose configuration is important in overall DNA structure [7]. Different binding modes process cellular machinery differently at the molecular target, i.e., binding to specific DNA sequence and/or structures. Understanding the features that contribute to enhance DNA binding by small ligands or metal complexes is crucial for the development of drugs targeted to DNA. The overall handedness of DNA molecule plays a major role in the recognition of DNA by chiral molecules due to two-pole complementary principle, “shape-selectivity” [8].

Furthermore, the use of stereochemistry can give clear insight into the mechanism of action allowing the discrimination between unspecific interactions, which are common to both enantiomers and specific contacts that give rise to enantioselectivity. Owing to this, there is a large demand for enantiomeric pharmaceuticals that have proven to be more efficacious, exhibiting less systemic toxicity and also possess high specificity. Chiral molecules therefore, play a critical role in the exploitation of three–dimensional space at the target site and regulate stereoselectivity in a highly organized fashion and were assumed to be therapeutically active as most of the biotargets of drugs are chiral in nature. Effect of chirality was carried out on an anticancer drug, Daunorubicin, the
REVIEW

Brassinosteroids and their role in response of plants to abiotic stresses

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Abstract

Brassinosteroids (BRs) are polyhydroxylated steroidal plant hormones that play pivotal role in the regulation of various plant growth and development processes. BR biosynthetic or signaling mutants clearly indicate that these plant steroids are essential for regulating a variety of physiological processes including cellular expansion and proliferation, vascular differentiation, male fertility, timing senescence, and leaf development. Moreover, BRs regulate the expression of hundreds of genes, affect the activity of numerous metabolic pathways, and help to control overall developmental programs leading to morphogenesis. On the other hand, the potential application of BRs in agriculture to improve growth and yield under various stress conditions including drought, salinity, extreme temperatures, and heavy metal (Cd, Cu, Al, and Ni) toxicity, is of immense significance as these stresses severely hamper the normal metabolism of plants. Keeping in mind the multifaceted role of BRs, an attempt has been made to cover the various aspects mediated by BRs particularly under stress conditions and a possible mechanism of action of BRs has also been suggested.

Additional key words: antioxidant system, drought, heavy metals, high temperature, low temperature, oxidative stress, photosynthesis.

Introduction

Plants constantly regulate their developmental and physiological processes in response to various internal and external stimuli. Studies have indicated that biological processes are integrated by multiple hormonal signals, and stresses induce the activities of different hormonal signaling pathways in plants (Teale et al. 2008). Out of the recognized categories of plant hormones, much attention has been focused on auxins, cytokinins, gibberellins, abscisic acid, and ethylene. Furthermore, brassinosteroids (BRs) are a group of steroidal hormones that play pivotal roles in wide range of developmental phenomena including cell division and cell elongation in stems and roots, photo-morphogenesis, reproductive development, leaf senescence, and also in stress responses (Choudhary et al. 2012).

The identification of plant endogenous steroidal hormones is the result of nearly 30 years of efforts to identify novel growth-promoting substances present in pollen grains of different plant species (Steffens 1991). Mitchell et al. (1970) showed that the growth stimulating activity was found in the organic solvent extract of pollen from *Brassica napus* and the unidentified active compound was named as brassin. The specific growth promoting effects of the brassin have been reflected in many bioassays including the bean second-internode bioassay (Mandava 1988). Based on their ability to cause marked changes in growth and differentiation at low concentrations, Mitchell et al. (1970) proposed that brassins constituted a new family of plant hormones known as brassinosteroids (BRs). Further work demonstrated that brassinosteroids not only induce stem elongation, they also increase total biomass and yield.

Although brassinosteroids were known to be endogenous regulators that induce dramatic growth
Synthesis, biological screening of novel long chain derivatives of 1,3-disubstituted-1H-pyrazol-5(4H)-one and 2-substituted-3H-1,4-phthalazin-1,4-dione: Structure-activity relationship studies

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Abstract The main purpose of this study is to synthesize novel heterocyclic derivatives of fatty acids which are also biologically important. The simple, efficient and one-pot synthesis of two novel series of 1-long chain alkanoyl/alkenoyl/hydroxyalkenoyl-3-methyl-1H-pyrazol-5(4H)-ones 2(a–e) and 2-long chain alkenoyl/hydroxyalkenoyl-3H-phthalazin-1,4-diones 3(b–e) is achieved by the reaction of ethylacetoacetate/phthalic anhydride and long chain alkyl/alkenyl/hydroxyalkenyl hydrazides 1(a–e). Although some methods are available for the synthesis of phthalazindiones and pyrazolones, the development of a new synthetic method for the efficacious build up of heterocycles (phthalazindiones and pyrazolones) substituted with long alkanoyl/alkenoyl/hydroxyalkenyl chain is an interesting challenge in the field of synthesis of novel compounds of fatty acids that includes heterocyclization and derivatization of fatty acids. Compounds 2(a–e) were synthesized by the cyclization reaction between ethylacetoacetate and long alkyl/alkenyl/hydroxyalkenyl chain hydrazides 1(a–e). Compounds 3(b–e) were synthesized by the reaction of phthalic anhydride and long alkenyl/hydroxyalkenyl chain hydrazides 1(b–e) in absolute ethanol/glacial AcOH. Structures of all the newly synthesized compounds have been elucidated by means of IR, 1H NMR, 13C NMR and MS. Newly synthesized compounds were evaluated for in vitro antibacterial and antifungal activities and their structure–activity relationship studies have been carried out.

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Original Research Paper

ANTIOXIDANT POTENTIAL OF SOME MEDICINAL PLANTS
(Ocimum sanctum, Azadirachta indica and Nigella sativa)

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ABSTRACT

The extracts of leaf, stem and root of Ocimum sanctum, Azadirachta indica and Nigella sativa, were used in the comparative study of total carotene, flavonoids, phenolics and total antioxidant potential. Leaves of Ocimum sanctum, exhibited maximum total carotene content (2.17±0.272 mg /gram fresh weight ±S.D), while root of Nigella sativa exhibited the lowest (0.29±0.088 mg /gram fresh weight ±S.D). Flavonoids were found to be maximum in the leaf of Nigella sativa (4.93±0.702 mg equivalent QE /gram of tissue ±S.D), while minimum in the root of Azadirachta indica (0.16±0.033 mg equivalent QE /gram of tissue ±S.D). A trend similar to total carotene content was exhibited by phenolics with (34.9±2.427 mg equivalent GA /gram of tissue ±S.D) in the leaf of Ocimum sanctum as maximum and (6.85±2.005 mg equivalent GA /gram of tissue ±S.D) in the root of Nigella sativa as minimum. Total antioxidant potential was observed to be maximum in the root of Ocimum sanctum (0.69±0.013 mM equivalent ascorbic acid/g tissue ±S.D), while minimum was recorded in the leaf of Nigella sativa (0.22±0.028 mM equivalent ascorbic acid/g tissue ±S.D). The variability when analyzed statistically by two way ANOVA it was found significant with (P-value < 0.05). The present study showed Ocimum sanctum to be an excellent source of antioxidants which can be utilized for therapeutic purposes. However, the specific plant part need to be optimally utilized for specific pharmaceutical/neutralcatical formulation.

Keywords: Antioxidant, Azadirachta indica, Carotene, Flavonoid, Nigella sativa, Ocimum sanctum, phenolics.

INTRODUCTION

Plants have been exploited as a substitute remedy for the treatment of variety of diseases ever since beginning of human civilization. In recent years focus has shifted from chemical drugs to exploitation of remedial plants as curative agent to treat numerous stress linked disorders due to production of free radicals. Free radicals are groups or atoms having one unpaired electron, which makes them extremely reactive. The potentially reactive derivatives of oxygen are acknowledged as reactive oxygen species (ROS; e.g., hydrogen peroxide, superoxide anions, hydroxyl, and nitric oxide radicals), which bring on oxidative damage to a variety of biomolecules including lipids, proteins and DNA etc. Free radical mediated damage may cause various diseases such as cancer, diabetes mellitus, arthritis, atherosclerosis, and neurodegenerative diseases, inflammatory diseases and also leads to ageing process (Halliwell and Gutteridge, 1985). In order to guard against free radicals, organisms are gifted with endogenous (superoxide dismutase, catalase, glutathione reductase / peroxidase) and exogenous (vitamins E and C, β-