Synthesis of nickel oxide nanoparticles for the assessment of antioxidant and antibacterial potentials: concentration and time dependent approach

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Abstract

Present investigation was aimed to evaluate the antioxidant and antimicrobial actions of chemically synthesized nickel oxide (NiO) nanoparticles at different concentration and time. The evaluation of different parameters was done on day 1, 30 and 60 after preparations of suspensions of different concentrations of NiO nanoparticles. NiO nanoparticles synthesized in this study were of 192.6 nm average size with a polydispersity index of 0.482. The quenching of different free radicals (DPPH, ABTS, superoxide anion and hydrogen peroxide radicals) by NiO nanoparticles was observed in concentration dependent manner. NiO nanoparticles of day 1 showed more percent inhibitions of free radicals and lesser IC_{50} values as compared to day 30 and 60. The antioxidant and antibacterial potentials of NiO nanoparticles suspensions decreased to some extent after their storage for 60 days.

Both the bacterial strains i.e. S. aureus and E. coli were susceptible for the action of NiO nanoparticles. The MIC and MBC values revealed that action of NiO nanoparticles was more against S. aureus than E. coli. In conclusion, the potent antioxidant potential and antibacterial actions shown by NiO nanoparticles make it promising candidate and might lead to a new insight in several fields like medical sciences, veterinary sciences, agriculture etc.

Keywords*:* Nickel oxide nanoparticles, Antioxidant, Antibacterial.

Introduction

Nanobiotechnology and nanomedicine have developed tremendously in recent few years in the field of nanoscience that utilize therapeutic nanoparticles for various biomedical applications. Nanobiotechnology is mainly focused on the synthesis and applications of nanoparticles which have played major role in the development of diverse fields like medical, agriculture, drug delivery, food, cosmetics etc. Principally, nanoscale matters differ from microscale matters in various properties like surface to volume ratio, mechanical, magneto-optical, chemical etc. which make them as a potential candidate in several biomedical appliances.

In recent years, emergence of antimicrobial resistance at global level has become a serious concern to the living organisms. Number of persons suffering from multidrug-resistant infections has increased tremendously. The indiscriminate use of traditional antibiotic treatment for bacterial infections is mainly associated with antibiotic resistance.

Staphylococcus aureus (*S. aureus*) and the *Escherichia coli* (*E. coli*) are considered to be the common key pathogens in biomaterial associated infections[5]. All over the globe, scientific communities are working at their best to develop novel strategies to combat the antibiotic resistance and reduce the incidences of development and spread of these infectious diseases[1, 27].

Additionally, lack of cost-effective and safe drinking water has also laid the human societies in danger and forces them to develop novel materials with new approaches to conquer this extreme nuisance[24]. Moreover, many disorders or diseases in humans and animals like cancer, diabetes, ulcerative colitis, aging, cardiovascular disease, atherosclerosis, mild cognitive impairment, Alzheimer's disease, neural disorders, Parkinson's disease, alcohol induced liver disease etc. are associated with the free radicals[25].

The antioxidants, natural and synthetic, scavenge the free radicals produced during various biological processes and thus, play a major role in the biosystems. The assessment of antioxidant property of a compound provides an insight about the behavior and interaction of material with biomolecules inside a living system. The investigation of antioxidant properties of nano sized materials has become one of the important basic studies in nanotechnology.

In last few decades, metal oxide nanoparticles have gained tremendous popularity with the ongoing developments on the interface of nanoscience for the development of novel and efficient strategies in biomedicine.

Metal oxide nanoparticles have interesting physio-chemical, electronic and optical characteristics properties for a broad range of appliances. Nickel oxide (NiO) nanoparticles have recently attracted wide attention due to their several uses in industry as well as academia. NiO nanoparticles have varieties of significant uses in energy storage devices, drug delivery, magnetic resonance imaging etc.[10,18].

NiO nanoparticles also have many novel properties compared to their bulk counterpart and are widely used in different areas in comaparison to its bulk form[20,26]. Only scanty of literature is available on antioxidant property and antimicrobial potentials of NiO nanoparticles especially after storage of these particles for different time period.

As, nano NiO is another very important strategic material used in biosystems, so it is important to study its potency for antioxidant and antimicrobial potentials with the aim to develop promising antioxidant and antimicrobial candidate. In our previous studies, we synthesized the NiO nanoparticles and evaluated acoustical parameters for different industrial applications. In present study, the NiO nanoparticles were synthesized and evaluated for their antioxidant and antibacterial potentials.

Materials and Methods

Chemicals used: Various types of analytical grade chemicals like nickel nitrate and sodium hydroxide were used for the synthesis of NiO nanoparticles and purchased from Sigma Aldrich, USA. Other analytical grade chemicals like 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, 2,2-diphenyl-1-picryl hydrazyl (DPPH), nitroblue tetrazolium (NBT), nicotineamide adenine dinucleotide hydrogen salt (NADH), phenazine methosulphate (PMS), disodium hydrogen phosphate, ascorbic acid, dimethyl sulfoxide (DMSO), potassium dihydrogen phosphate, 2- deoxy-D-ribose, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), ethylene diamine tetramine (EDTA), ferric chloride ($FeCl_3$), hydrogen peroxide (H_2O_2) etc. were procured from SRL for antioxidant assays.

For the antimicrobial activity, *S. aureus* (MTCC 1430) and *E. coli* (MTCC 2127) were the two bacterial strains used in this study. Tetracycline and gentamicin were used as standard antibiotics and procured from Sigma Aldrich, USA. Nutrient broth (NB), nutrient agar (NA) etc. were purchased from SRL and they were also of analytical grade. All chemicals were used as received without further purification.

Preparation of NiO nanoparticles: NiO nanoparticles were synthesized by the precipitation of nickel salt in alkaline medium. Briefly, 0.25 M nickel nitrate (aqueous solution) and 1.25 M sodium hydroxide (aqueous solution) were reacted at 55 ^oC with constant stirring for the synthesis of NiO nanoparticles. The whole mixture was kept on magnetic stirred for 2 h and the pH of the mixture was adjusted to 12. The suspension of green color formed was centrifuged followed by filtration and washed to get NiO precipitates. Thereafter, these precipitates were calcined at 600 °C for 3 h in ambient atmosphere to obtain nano NiO particles.

The particle size of synthesized NiO nanoparticles was determined by Malvern Instruments Zetasizer Nano-ZS instrument. Different concentrations of NiO nanoparticles were dispersed in DMSO by employing ultrasonication for further studies like *in vitro* antioxidant potentials against various types of free radicals and antimicrobial activity. In antimicrobial assay, *in vitro* minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of synthesized NiO nanoparticles were determined against *S. aureus* and *E. coli* bacterial strains. The suspension of different concentrations of NiO nanoparticles was kept at room temperature and further studied for these *in vitro* parameters on day 30 and 60 in order to evaluate the storage stability.

Antioxidant activity studies of NiO nanoparticles:

A. DPPH radical scavenging potential: Spectrophotometric procedure was used to determine the DPPH radicals scavenging potential of NiO nanoparticles as per the method described by Hsu et al[9] and Kant et al.[14] Different concentrations ranging from 2.5 to 160.0 µg/ml were used for free radical scavenging activity of NiO nanoparticles. Briefly, NiO nanoparticles (different concentrations) were added to DPPH solution and DPPH solution without NiO nanoparticles was used as control solution. Solutions were kept at room temperature (22 ± 3°C) in the dark for 30 minutes and the absorbance of the samples and control solutions was determined at 517 nm.

The ascorbic acid was used as standard antioxidant and evaluated in parallel similarly as done for NiO nanoparticles. Methanol was used as a blank. Control sample was prepared containing the same amount of methanol and DPPH without nanoparticles. The sample concentration providing 50% inhibition (IC_{50}) was calculated and reported as mean ± SE. The % DPPH radical scavenging activity was calculated by employing the following formula:

% DPPH radical scavenging activity = [1– (A_{517}nm sample / A_{517}nm control)] x 100

B. ABTS radical scavenging potential: The methods of Re et al[17] and Kant et al[14] were used for the total antioxidant activity of NiO nanoparticles on the basis of $ABTS^{\bullet+}$ scavenging assay. NiO nanoparticles were evaluated in concentration ranging from 2.5 to 160.0 µg/ml. Briefly, diluted $ABTS^{\bullet+}$ solution having 0.750 at 734 nm absorbance reading was used for antioxidant activity of different concentrations of NiO nanoparticles on different days.

The radical scavenging capacity was performed by mixing NiO nanoparticles (different concentrations) with $ABTS^{\bullet+}$ solution. After proper mixing, the absorbance was recorded at 734 nm after 3 min. $ABTS^{\bullet+}$ solution without nanoparticles was used as control solution.

The ascorbic acid was used as standard antioxidant and evaluated in parallel similarly as done for NiO nanoparticles. The percentage of inhibition of $ABTS^{\bullet+}$ radicals at different concentrations was estimated as follows:

% $ABTS^{\bullet+}$ inhibition = [1 – (A_{734}nm Sample/ A_{734}nm Control)] x 100

C. Superoxide anion radical scavenging potential: The methods described by Nishikimi et al[15] and Kant et al[14] were used for assessing the superoxide anion radical-scavenging potential of NiO nanoparticles. The NiO nanoparticles concentrations ranging from 2.5 to 160.0 µg/ml were used for superoxide anion radical scavenging activity. Briefly, the reaction mixture contained 1 ml of NBT solution (156 µM prepared in phosphate buffer, pH-7.4), 1ml of NADH solution (468 µM prepared in phosphate buffer, pH-7.4) and 0.5 ml diluted sample of different concentrations fraction.

Finally, acceleration of the reaction was carried out by adding 100 µL PMS solution (60 µM prepared in phosphate buffer, pH -7.4) to the mixture. The reaction mixture and control sample (without nanoparticles) were mixed properly and incubated at 25ºC for 5 min and absorbance at 560 nm was measured.

The ascorbic acid was used as standard antioxidant and evaluated in parallel similarly as done for NiO nanoparticles. Percentage inhibition of the superoxide anion radicals was calculated using the following equation:

% superoxide radical scavenging activity = [1– (A_{560}nm sample / A_{560}nm control)] x 100

D. Hydrogen peroxide radical scavenging potential: Total reducing power activity of samples was investigated according to the method described by Jayaprakasha et al[12]. Briefly, a solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (pH 7.4). One ml of various concentrations ranging from 2.5 to 160.0 µg/ml of NiO nanoparticles and standard (ascorbic acid) were added to 2 ml of hydrogen peroxide solution in PBS. Thereafter, the absorbance was measured with a spectrophotometer at 230 nm after 10 min.

% Hydrogen peroxide radical scavenging activity = [1– (A_{230}nm sample / A_{230}nm control)] x 100

Estimation of minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of NiO nanoparticles: The MIC and MBC of NiO was determined against *S. aureus* and *E. coli* bacterial strains by tube dilution method. Briefly, both bacterial strains were used overnight incubated and suspension were aseptically inoculated (about 10^6 CFU/ml) to 10ml tube nutrient broth medium. Ten dilutions of NiO nanoparticles were prepared (10, 20, 40, 60, 80, 100, 140, 180, 220 and 260 µg/ml) in DMSO. Tests were performed in triplicate for each concentration and for each bacterial strain.

The inoculated sets were incubated at 37ºC overnight. After incubation period, the visible turbidity in each tube was investigated. The lowest concentration with no turbidity was considered as the MIC for the tested strain. The tubes showing no turbidity were cultured on nutrient agar plates and incubated at 37ºC overnight. Bacterial colonies growth was checked and the concentration that showed no growth was considered as the MBC for the tested strain.

Results and Discussion

NiO nanoparticles synthesized in this study were of 192.6 nm average size with a polydispersity index of 0.482. The average size of the nanoparticle suspension on day 30 and 60 was 221.7 nm and 268.2 nm respectively. In our previous study, NiO nanoparticles synthesized by same method were of spherical shape and average size by TEM studies in the range of range 30-47 nm (data under publication).

The different concentrations of ascorbic acid and synthesized NiO nanoparticles showed the inhibition of the different *in-vitro* free radicals i.e. DPPH (Fig. 1), ABTS (Fig. 2), superoxide anion (Fig. 3) and H_2O_2 radicals (Fig. 4) in a concentration dependent manner on different days. The concentrations showing the 50% inhibition of free radicals are known as IC_{50} and the IC_{50} values of ascorbic acid and synthesized NiO nanoparticles for the total antioxidant activity, free radical scavenging assay, superoxide radical scavenging activity and reducing power activity are presented in table 1.

Free radicals are of different chemical entities with one or more unpaired electrons, which are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. These free radicals are formed inside the system and are highly reactive and potentially damaging the short lived chemical species.

Table 1
IC_{50} values (µg/ml) of NiO nanoparticles against DPPH, ABTS, superoxide anion and hydrogen peroxide radicals on different days.

Parameters	Ascorbic acid	NiO nanoparticles		
		Day 1	Day 30	Day 60
Free radical scavenging activity (DPPH) (µg/ml)	57.16±1.29	71.48±3.98	88.16±2.91	98.18±2.06
Total antioxidant activity (ABTS) (µg/ml)	95.81±2.66	128.28±4.50	136.76±4.01	140.46±3.98
Superoxide radical scavenging activity (µg/ml)	80.31±3.40	151.15±4.09	159.22±5.26	177.16±4.78
Reducing power activity (µg/ml)	72.08±4.15	137.37±3.80	145.10±4.29	161.33±3.81

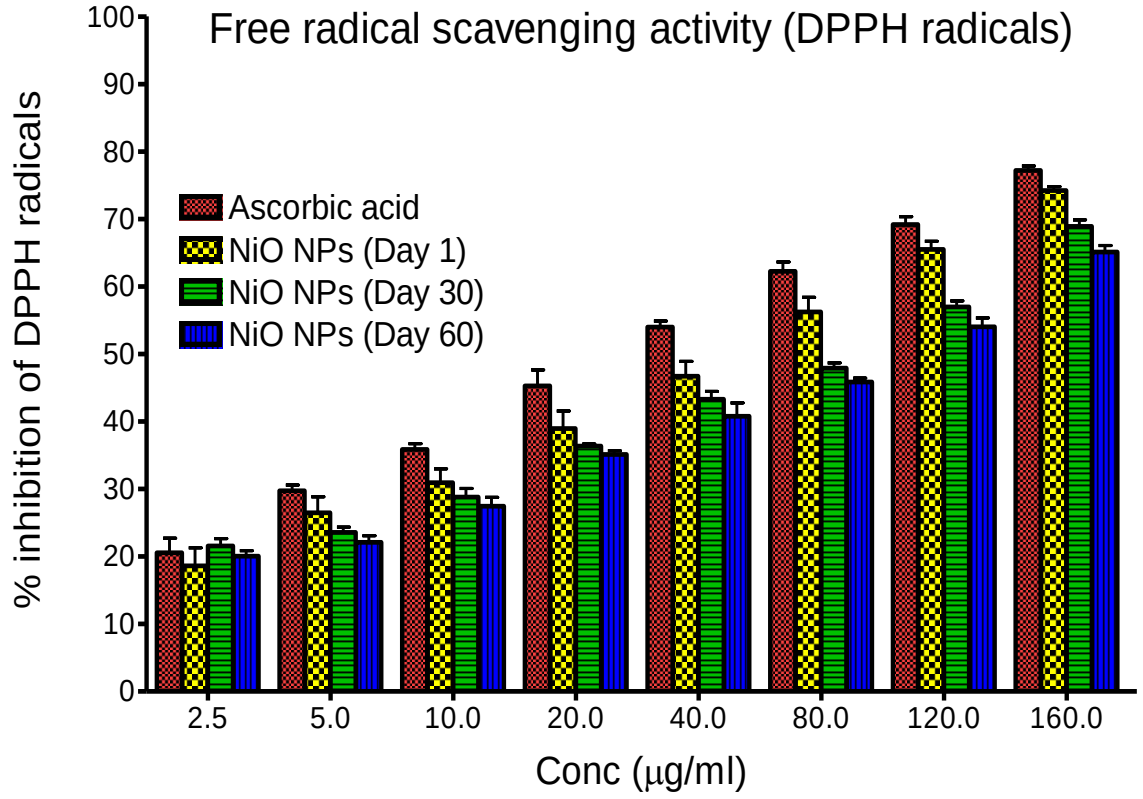

Fig. 1: DPPH radicals scavenging activity of NiO nanoparticles (NPs) on different days at different concentrations. Results are expressed as mean ± SE (n = 3)

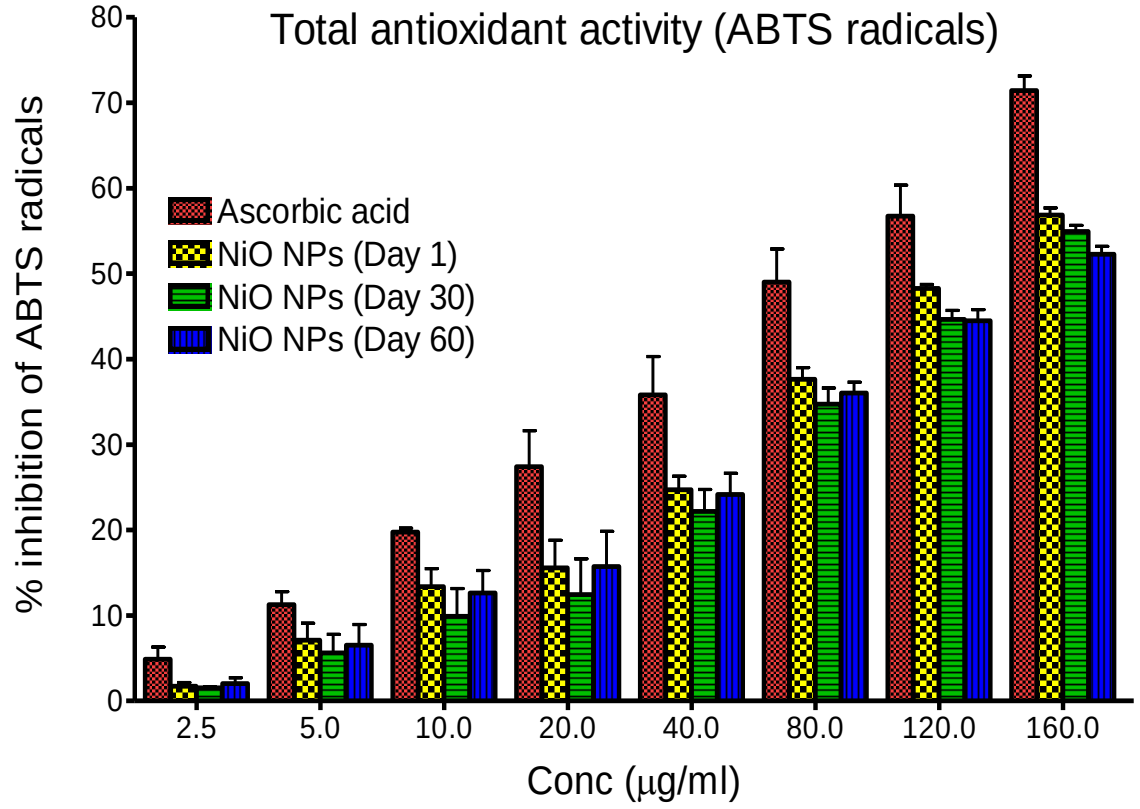

Fig. 2: ABTS radicals scavenging activity of NiO nanoparticles (NPs) on different days at different concentrations. Results are expressed as mean ± SE (n = 3).

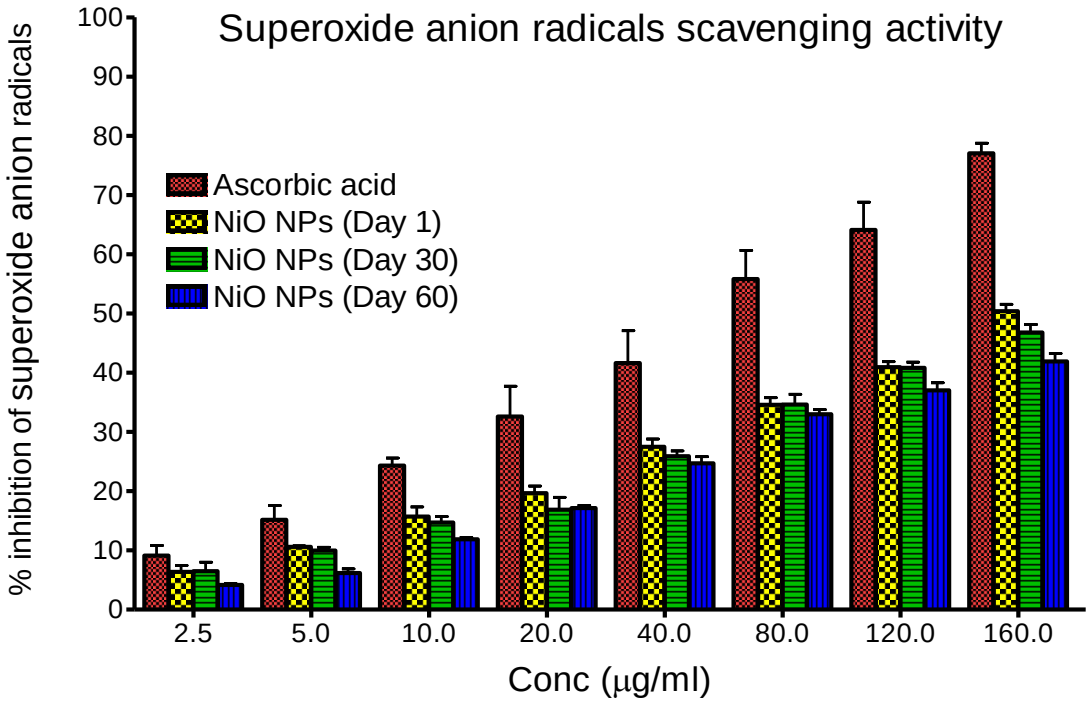

Fig. 3: Superoxide anion radicals scavenging activity of NiO nanoparticles (NPs) on different days at different concentrations. Results are expressed as mean ± SE (n = 3).

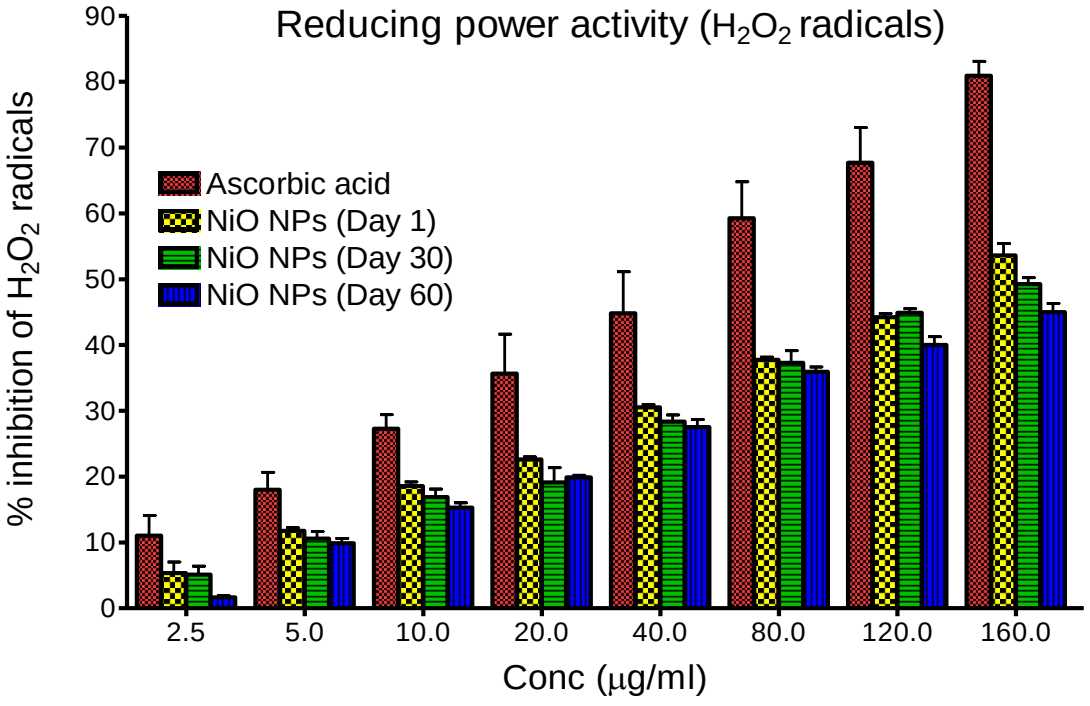

Fig. 4: H_2O_2 radicals scavenging activity of NiO nanoparticles (NPs) on different days at different concentrations. Results are expressed as mean ± SE (n = 3)

There is continuous production of these radicals in the human and animal body due to their role in detoxification, chemical signaling, energy supply and immune function.

DPPH is a stable free radical, which accepts an electron or hydrogen radical to become a stable diamagnetic molecule due to their hydrogen donating ability. DPPH method has been widely used as a gold standard to investigate the free radical scavenging activity. The method of DPPH radical is used to evaluate antioxidant activities in a shorter period of time as compared to other methods[21]. The stabilized DPPH produces intense violet colour and the loss of this violet

colour occurs in presence of antioxidant due to hydrogen donating capacity of antioxidants.

The results of this study showed marked quenching ability of NiO nanoparticles for DPPH-free radical in a concentration-dependent manner and there was little reduction in the scavenging activity after 30 and 60 days storage of NiO suspensions.

Previous researchers have also showed DPPH radical scavenging potential of chemically synthesized NiO nanoparticles[19]. The IC_{50} value of NiO nanoparticles against DPPH radicals on day 1 (71.48±3.98) was less as compared to day 30 (88.16±2.91) and 60 values (98.18±2.06). DPPH-free radical scavenging by NiO nanoparticles was also supported by its ABTS radical scavenging activity. ABTS is a chemically produced radical which decolorizes in its non-radical form in the presence of antioxidants and is often used for screening complex mixtures such as plant extracts, beverages, biological fluids and other antioxidants.

In present study, marked ABTS radical scavenging activity of NiO nanoparticles was observed in a concentration-dependent manner. The scavenging activity of NiO nanoparticles was slightly decreased on its storage. The IC_{50} value of chemically synthesized NiO nanoparticles on day 1, 30 and 60 against ABTS radicals was 128.28±4.50, 136.76±4.01 and 140.46±3.98 respectively.

Superoxide anion radical is a reduced form of molecular oxygen implicated in the initiating of oxidation reactions associated with aging and lipid peroxidation.

In this study, NiO nanoparticles markedly scavenge the superoxide anion radicals in concentration dependent manner and reduction in the scavenging activity of NiO nanoparticles against superoxide anion radicals was also observed on storage of suspension of NiO nanoparticles.

The IC_{50} value of NiO nanoparticles against superoxide anion radicals on day 1 (151.15±4.09) was less as compared to day 30 (159.22±5.26) and 60 (177.16±4.78) values. The antioxidant activity generally also has a mutual correlation with reducing effect. It is known that hydrogen peroxide is toxic and induces cell death *in vitro* and can attack many cellular energy production systems deactivating the glycolytic enzyme glyceral- dehyde-3-phosphate dehydrogenase[2]. Scavenging of hydrogen peroxide by different concentrations of NiO nanoparticles in present study was observed in concentration dependent manner and reduction in the scavenging activity of NiO nanoparticles against hydrogen peroxide was also observed on storage of suspension of NiO nanoparticles.

The IC_{50} value of NiO nanoparticles against hydrogen peroxide on day 1 (137.37±3.80) was also less as compared to day 30 (145.10±4.29) and 60 (161.33±3.81) values. In present study, ascorbic acid also showed inhibitions of ABTS, DPPH, superoxide anion and H_2O_2 radicals in concentration dependent manner and it was more potent in scavenging these radicals as compared to the NiO nanoparticles synthesized in this study. Additionally, IC_{50} value of ascorbic acid was lesser than NiO nanoparticles against these radicals. It is known that lower are the IC_{50} values, greater is the hydrogen donating ability and thus the antioxidant activity of the free radical scavengers.

The values of MIC and MBC for the synthesized NiO nanoparticles on different days against *S. aureus* and *E. coli* bacterial strains are presented in table 2. The NiO nanoparticles showed antibacterial actions against both the bacterial strains which revealed its powerful broad spectrum anti-bacterial activity.

Generally, the microbial inhibition potential was higher against the gram-negative bacteria than the gram-positive bacteria due to the absence of a peptidoglycan layer in the gram-negative bacteria, whereas the gram-positive bacteria had a thick peptidoglycan layer and the fungi were more resistant to environmental conditions and had a strong chitinous membrane layer for protection[3,8].

However, the values of MIC and MBC of NiO nanoparticles in our study showed that their action was more against the *S. aureus* than *E. coli*. This might be due to their minute size and stability, the NiO-NPs showed enhanced antibacterial activity in the present study. The antimicrobial activity of a nanoparticles depends on the size, structure and composition. Smaller size generally improved the dispersibility and diffusion into the intracellular matrix and hampered the intracellular Ca^{2+} absorption, which resulted in cell death.

Smaller sized particles initiated electrostatic interactions between the nickel ions released from the NiO nanoparticles and the cell membrane of the microbes which resulted to damage of cell membrane[4].

Table 2
The MIC and MBC values (µg/ml) of NiO nanoparticles (NPs) against *S. aureus* and *E. coli* on different days.

Bacteria	MIC			MBC		
	NiO NPs (Day 1)	NiO NPs (Day 30)	NiO NPs (Day 60)	NiO NPs (Day 1)	NiO NPs (Day 30)	NiO NPs (Day 60)
S. aureus	80.0 µg/ml	80.0 µg/ml	80.0 µg/ml	180.0 µg/ml	180.0 µg/ml	220.0 µg/ml
E. coli	100.0 µg/ml	100.0 µg/ml	100.0 µg/ml	220.0 µg/ml	220.0 µg/ml	260.0 µg/ml

The damaged cell membrane is more sensitive to further interactions and permits for the penetration of nanoparticles and leakage of the intracellular organelles. These sequential steps enhance the inhibitory activity of the nanoparticles. There are various mechanisms of actions through which metal oxide nanoparticles induce bacterial toxicity like oxidative stress, lipid peroxidation, cell membrane lysis, enzyme inhibition, proteolysis etc.[6]. At physiological pH, the overall charge of the bacterial cell is negative due to the dissociation of excess carboxylic groups at the cell surface[23].

So, NiO became electrostatically bound to the negative cell surface, as NiO has a positive surface charge. This further hinders the bacterial activity. Bacterial cell became inactive and dead followed by lysis after the penetration of NiO into the cell[16]. Therefore, in present study, NiO nanoparticles might be responsible for damaging the bacterial cell membrane followed by cell lysis by the direct contact.

It was also evident in present study that the MBC value of NiO nanoparticles for *S. aureus* and *E. coli* increased on its storage for two months. This might be due to its increased size because of agglomerations of the nanoparticles during the storage, which led to decreased actions of the nanoparticles. However, there was no effect on storage stability on the MIC values for both bacterial strains. So, storage of NiO nanoparticles suspension for 60 days at room temperature did not produce marked reduction in the antibacterial potentials, and it might be suggested that the suspension of these Nanoparticles is stable at room temperature for 60 days.

The chances of occurrence of bacterial resistance against metal oxide nanoparticles are very rare and these compounds may be considered safe potential alternatives antimicrobial for clinical applications[11,13]. Stability at high temperatures as well as pressures without loss of medicinal properties and safety can give upper hand to NiO nanoparticles for antimicrobial activity in comparison to organic compounds[7, 22].

Prevention is better than cure, a well known phrase in biomedical sciences. Therefore, NiO nanoparticles application/coating on large active surface area, uniforms, bed linen, on cellulose bandages, medical equipment, paintings etc. may be useful preventive measure to combat bacterial/microbial infections. This might be useful in reducing bacterial contamination, mortality, treatment of costs etc. The utility of NiO nanoparticles in commercial paintings may kill the airborne pathogens with high efficiency and can also be utilized in wastewater treatment. The antioxidant and antibacterial potential may be employed in the field of agriculture as an alternative to environmentally toxic chemical pesticides.

Conclusion

In conclusion, the potent antioxidant potential (against DPPH, ABTS, superoxide anion and hydrogen peroxide radicals) and antibacterial actions shown by NiO nanoparticles make it promising candidate and might lead to a new insight in several fields like medical sciences, veterinary sciences, agriculture etc.

Finally, the use of NiO nanoparticles may be extended to food industry, water purification, textile industry, paint industry, sewage treatments etc.

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