

## Synthesis, characterization and concentration dependant antibacterial potentials of nickel oxide nanoparticles against *Staphylococcus aureus* and *Escherichia coli*

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Bacterial resistance to antibiotic treatment is a major emerging clinical and public health issue across the globe. Advancements in the field of metal oxide nanomaterials in the last few years have improved the potential of metal oxides in different applications. Metal oxides, of which, nickel oxide (NiO) is one, also possess antibacterial activities. This investigation was planned to synthesize NiO nanoparticles to study their antibacterial potential in comparison with bulk NiO and standard antibiotics at different concentrations. Synthesis and characterization of NiO nanoparticles was done by standard procedures. The antibacterial potentials of different compounds were determined at different concentrations against *S. aureus* and *E. coli*. The diameter of zone of inhibition showed that the antibacterial effect of NiO nanoparticles against *S. aureus* was better than *E. coli* at the same concentration. The concentration-dependent effect of NiO nanoparticles was observed from 0.125 to 128  $\mu\text{g/ml}$ . The effect of NiO nanoparticles was markedly better than bulk NiO at all concentrations. Tetracycline and gentamicin did not show effect below 1.0  $\mu\text{g/ml}$  and 2.0  $\mu\text{g/ml}$ , respectively. The activity index and fold increase of NiO nanoparticles were both higher than 1 and positive, with respect to tetracycline, gentamicin and bulk NiO against *S. aureus* and *E. coli* at all the tested concentrations. In conclusion, the NiO nanoparticles seemed to be a more potent antibacterial agent than their bulk form, tetracycline and gentamicin, and in future, their applications may be extended in biomedical field and other areas to reduce microbial infections and incidences of antibacterial resistance.

**Keywords:** nickel oxide nanoparticles, *S. aureus*, *E. coli*, Antibacterial activity, activity index, fold increase.

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### 1. Introduction

The growth and proliferation of bacteria can occur fruitfully anywhere. Bacterial infections, due to both gram-positive as well as gram-negative bacterial strains, are considered as serious health problems worldwide. Bacterial infectious diseases cause huge morbidity and mortality, thus, there is need to develop effective antimicrobial agents. Over the years, different infections resulting from society as well as hospital atmosphere have been controlled by the use of antibiotics [1]. Exposure of bacteria to several environmental variations such as temperature, osmolarity, radiation, toxins, and limited nutrition make them capable to survive in diverse environments by developing various approaches. Bacterial resistance to antibiotic treatment is emerging a major clinical and public health issue across the globe, which is also a serious concern for physician. Antimicrobial resistance is mainly due to indiscriminate use of antibiotic medications, lack of new strategies for antibacterial resistance development etc. [2]. This problem has added financial burden to the healthcare system. It is also predicted that antibacterial resistance will achieve worldwide epidemic proportions by 2050 and account for approximately 10 million casualties [3, 4]. In view of its global importance, appropriate strategies and actions are needed from governmental sectors, industry, healthcare professions, farmers, society etc. Bacteria can develop resistance to treatments by different pathways, like decreased influx and increased efflux mechanisms, alteration in binding target sites, production of some enzymes etc. Development of more efficient antibacterial agents to overcome the problems of bacterial mutation, antibiotic resistance, outbreaks of pathogenic strains, etc. is demanded in the present era.

Different metals and metal oxides have been used as an antimicrobial agents for thousands of years. Indians have also used copper and silver to preserve food and disinfect water [5]. However, after the discovery of antibiotics in the twentieth century, their antimicrobial properties in a variety of medical applications have rapidly diminished. Recent basic and applied research on various metal oxides has led to their broad scale application in various areas such as catalysis, in semiconductors, sensors, controlled release of drugs and as antimicrobial agents. Advancements in the field of nanobiotechnology in the last few years have also led to the development of various metal oxide nanomaterials, which are emerging as one of the new antibacterial agents. Studies on the synthesis, characterization and applications

of nanoparticles as an antimicrobial system is the latest area of interest in the biomedical and healthcare sectors. Nanoparticles are considered better antimicrobial agents than their bulk forms against drug-resistant pathogens due to size effect, doping effect, cost effective and stability, prolonged shelf-life etc. Recently, nanoparticles of many metal oxides like zinc oxide, copper oxide, iron oxide, cobalt oxide, cerium oxide etc. have shown various biological activities including antibacterial activity against Gram-positive and Gram-negative bacteria [6–9]. The bactericidal property by different metal oxide nanoparticles occurs by various mechanisms [10]. Additionally, nanoparticles of cobalt have shown better antibacterial efficacy than its bulk form against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) [11]. It has also been reported that silver nanoparticles stabilized with neomycin-chitosan has enhanced antimicrobial activity relative to the neomycin-chitosan system and silver nanoparticles alone [12].

Nickel oxide (NiO), a P-type semiconductor transition metal oxide, in bulk and nano size has received significant attention due to its wide range of applications in different fields [13]. The nano NiO possesses significantly different catalytic, optical, electronic, and magnetic properties than its bulk form due to quantum size and surface effects [14, 15]. Although, various investigations have documented the efficient applications and properties of NiO based materials, but the detailed studies on antimicrobial activity of NiO and its nano form are very limited and mostly lacking in comparison of antibacterial actions of NiO nanoparticles with respect to its bulk form as well as standard antibiotics. In view of this, the present investigation sought to prepare NiO nanoparticles to determine their antibacterial action against commonly present bacterial strains i.e. *S. aureus* and *E. coli*, and their comparison of antibacterial actions to bulk NiO and standard antibacterials (tetracycline and gentamicin) at different concentrations.

## 2. Materials and method

### 2.1. Materials used

The different chemicals of analytical grade were used for in this investigations. Nickel nitrate and sodium hydroxide were used for the synthesis of NiO nanoparticles and purchased from Sigma Aldrich, USA. Dimethyl sulfoxide (DMSO) was purchased from SRL. Tetracycline and gentamicin were procured from Sigma Aldrich, USA. The Muller-Hinton Agar (MHA), Muller-Hinton Broth (MHB) and nutrient broth (NB) were purchased from Hi-media, Mumbai, India and used for antibacterial studies. The *S. aureus* (MTCC 1430) and *E. coli* (MTCC 2127) gifted from the Department of Biotechnology, University of Jammu were revived using the nutrient broth (NB) and used two bacterial strains in this study.

### 2.2. Synthesis of NiO nanoparticles

Aqueous solution of 0.25 M nickel nitrate and 1.25 M sodium hydroxide with constant stirring at 55 °C were reacted for the synthesis of NiO nanoparticles. The complete mixture was magnetically stirred for 2 h at pH 12, forming a green precipitate of nickel hydroxide which was centrifuged, filtered and washed. Thereafter, they were calcined at 600 °C for 3 h to obtain NiO nanoparticles. The synthesized NiO nanoparticles at different concentrations were dispersed in DMSO by employing ultrasonication for further studies. There was no addition of stabilizer during any step in order to avoid its effects on the surface property of the nanoparticle as well as chemical contamination.

### 2.3. Characterization of NiO nanoparticles

The synthesized nanoparticles were characterized by using various techniques like particle size analyzer (PSA), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The particle size distribution (PSD) analysis of the synthesized nanoparticles was done by Malvern Instruments Zetasizer Nano-ZS instrument. The transmission electron microscope (Hitachi H-7500) was used to evaluate the size and morphology of nanoparticles. The surface morphology of the particles was observed by a scanning electron microscope (SEM-EDS) using SEM (JEOL JSM - 6390LV).

### 2.4. Assessment of antibacterial activity

**2.4.1. Determination of zone of inhibition by well-diffusion method.** Different concentrations of tetracycline, gentamicin, bulk NiO and synthesized NiO nanoparticles were used to evaluate their antibacterial potentials against the gram-positive i.e. *S. aureus* and gram-negative i.e. *E. coli* bacterial strains. The antibacterial activity was determined on Muller-Hinton agar by the agar well diffusion method [16]. The agar used for the study was seeded with overnight cultures of different bacterial strains ( $10^8$  cfu/ml) and then poured (12 ml) in petri plates. Five 6 mm wells of diameter were punched into the agar plates for testing antimicrobial activity of nanoparticles and other compounds. Prepared wells were also sealed with one drop of melted agar (0.8% agar) to prevent the leakage of nanoparticles and other compounds from the bottom of the wells. Using a micropipette, different dilutions of NiO nanoparticles, bulk NiO and antibiotics were poured onto each of five wells on all plates. After overnight incubation at 37 °C, the different

diameters of zone of inhibition were measured to quantify the antibacterial activity. In this study, DMSO was used as negative control, and tetracycline and gentamicin were used as positive control to compare the efficacy of the nanoparticles. The same experiment for all the dilutions was conducted in triplicate. The activity index and fold increase for the comparison of antibacterial potentials of NiO nanoparticles with bulk NiO and standard antibiotics was also determined by using values of zone of inhibition.

**2.4.2. Activity index for NiO nanoparticles.** The activity index was calculated by comparing the resultant zones of inhibition of NiO nanoparticles with the standard antibiotic or bulk NiO by using the following formula:

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the NiO nanoparticles}}{\text{Inhibition zone of the bulk NiO or standard antibiotic}}$$

**2.4.3. Fold increase for NiO nanoparticles.** Fold increase was also determined by comparing the resultant zones of inhibition of NiO nanoparticles with the standard antibiotic or bulk NiO. The fold increase was calculated by using the following equation:

$$\text{Fold increase(\%)} = \frac{b - a}{a} \cdot 100,$$

where  $a$  and  $b$  refer to the inhibition zones of antibiotic/ bulk NiO and NiO nanoparticles, respectively.

### 3. Results and discussion

The PSD of NiO nanoparticles is shown in Fig. 1A and it showed the overall z-average size of 178.6 nm with a polydispersity index of 0.239. This signified that the particle size distribution consisted of a single size mode devoid of aggregates. The TEM (Fig. 1B) and SEM (Fig. 1C) micrographs of NiO nanoparticles showed that the synthesized NiO nanoparticles have the average size of 40–55 nm and almost spherical shape. The SEM micrograph also demonstrated that the majority of the particles displayed little aggregation.

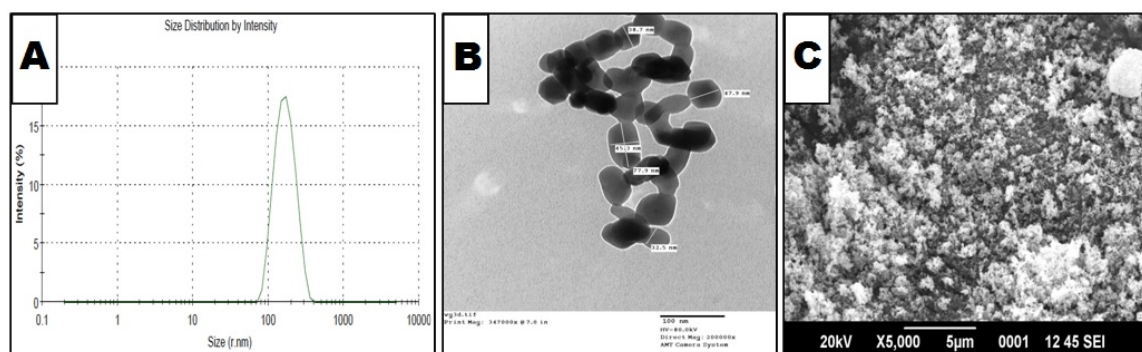


FIG. 1. (A) The particle size distribution (PSD) of NiO nanoparticles. Micrograph of TEM (B) and SEM (C) of NiO nanoparticles

Images of bacterial culture plates of present study evidently showed that tetracycline, gentamicin, bulk NiO and NiO nanoparticles produced zones of inhibition against *S. aureus* and *E. coli* in concentration dependent manners (Fig. 2). The mean diameters of zones of inhibition for different tested compounds at different concentrations are presented in Table 1. It has been previously reported that the inhibition of bacterial growth increases with an increase in concentration of nanoparticles [17]. Earlier, different nanoparticles have been studied for antimicrobial activities against human pathogenic bacteria such as *S. aureus* and *E. coli* [18, 19]. The synthesized NiO nanoparticles produced larger zone of inhibition against *S. aureus* in comparison to *E. coli* at the same concentration. It was revealed that NiO nanoparticles have stronger action against *S. aureus* than *E. coli*. These results are in agreement with the earlier studies in which *S. aureus* were more sensitive than *E. coli* to antibacterial actions of Ni nanoparticles [20]. In recent years, also, few studies have shown the antimicrobial actions of NiO nanoparticles prepared by different methods. Khalil et al [21] biologically synthesized NiO nanoparticles by using aqueous leaf extracts of *Sageretia thea*, and observed their antimicrobial properties by in vitro assays. Their study also revealed that the antibacterial activities of NiO nanoparticles were enhanced after UV illumination and *E. coli* as well as *B. subtilis* were the most susceptible strains. Srihasam et al., [22] produced NiO nanoparticles using *Stevia* leaf broth, which showed the antimicrobial actions strongly against gram-negative bacteria. The synthesized nanoparticles in their study ranged from 20 to 50 nm and were spherical in shape. Helan et al [23] prepared the NiO nanoparticles with the help of phytoconstituents present in the neem leaf and observed their antibacterial activity, which was concentration-dependent. In their study,

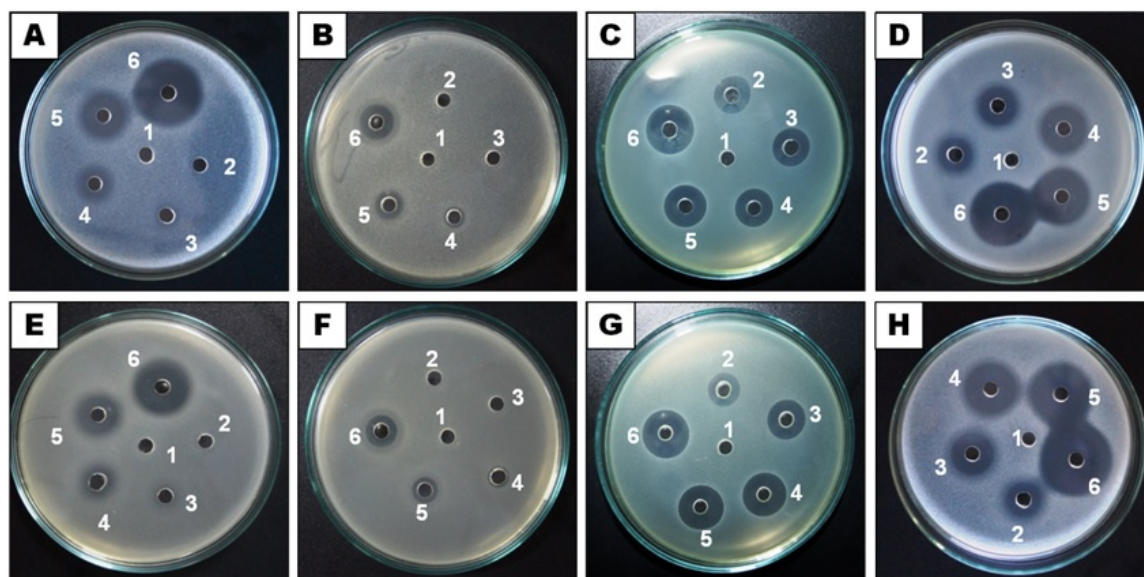


FIG. 2. Zone of inhibitions of tetracycline (A,E), gentamicin (B,F), bulk NiO (C,G) and NiO nanoparticles (D,H) against *S. aureus* (A-D) and *E. coli* as (E-H) at different concentrations i.e. (1) 0.00  $\mu\text{g/ml}$ , (2) 0.125  $\mu\text{g/ml}$ , (3) 0.5  $\mu\text{g/ml}$ , (4) 2.0  $\mu\text{g/ml}$ , (5) 16.0  $\mu\text{g/ml}$  and (6) 128  $\mu\text{g/ml}$

morphological analysis revealed oblong shape and 12 nm size of nanoparticles. Abbasi et al [24] also synthesized NiO nanoparticles by an environmentally-benign synthetic method using *Geranium wallichianum* as reducing and capping agent. The NiO nanoparticles in their study were of 21 nm size and nanoparticles showed antibacterial potential against different bacterial strains at different concentrations (700–21,875  $\mu\text{g/ml}$ ). In their study, the *B. subtilis* (MIC: 21,875  $\mu\text{g/ml}$ ) was the most susceptible organism, and *K. pneumonia* (MIC: 175  $\mu\text{g/ml}$ ) as well as *P. aeruginosa* (MIC: 175  $\mu\text{g/ml}$ ) were the least susceptible bacterial strains with respect to the action of NiO nanoparticles. Bhat et al [25] synthesized face-centered cubic structure of NiO nanoparticles with 30 nm average size by co-precipitation method. In their study, *K. pneumonia* and *B. subtilis* showed maximum zone of inhibition (15 mm) at 40 mg/ml concentration, and minimum inhibitory concentration 62.5  $\mu\text{g/ml}$ . In our present study, the antibacterial actions of synthesized NiO nanoparticles increased with an increase in concentration, and this concentration dependent action was observed only from 0.125 to 128  $\mu\text{g/ml}$ . It was observed in this study that the action of the nanoparticles decreased when the concentration of nanoparticles increased beyond 128  $\mu\text{g/ml}$ . This decreased action at higher concentrations was postulated to be due to the agglomeration of nanoparticles at higher concentrations. Some previous studies have also explained in their findings that nanoparticles possess some optimum concentration at which higher effects are noticed in comparison to more or less concentration [26]. Thus, the optimum concentration for NiO nanoparticles in the present study was 128  $\mu\text{g/ml}$ . Standard antibacterials i.e. tetracycline and gentamicin did not show zones of inhibition at lower concentrations i.e. below 1.0  $\mu\text{g/ml}$  for tetracycline and below 2.0  $\mu\text{g/ml}$  for gentamicin (Table 1) and seemed ineffective at lower concentrations. On the other hand, the bulk NiO and NiO nanoparticles used in this study were effective below these concentrations (Tables 1). It suggested that the range of inhibitory concentration for bulk and nano NiO was broader with respect to both tetracycline and gentamicin. The antibacterial effects of nano form of NiO was observed markedly better than bulk form at all tested concentrations of present study. Hence, NiO nanoparticles can provide antibacterial action for longer duration with less frequency of applications and may be considered as a substitute for tetracycline, gentamicin and bulk NiO in different fields in view of to diminish the occurrence of bacterial resistance.

The size, morphology and concentration of nanoparticles significantly influence their physical and chemical properties, which affect the degree of growth and survival of bacteria. Different previous studies have revealed that metal oxide nanoparticles between 1 and 100 nm size with different shapes are promising antimicrobial agents against infectious diseases [27]. The TEM micrograph in present study showed average size of 40–55 nm and spherical shape of synthesized NiO nanoparticles. So, nanoparticles synthesized in this study could be considered promising antimicrobial agents. The metal oxide nanoparticles induce bacterial toxicity through various mechanisms of actions like, oxidative stress, lipid peroxidation, cell membrane lysis, enzyme inhibition, proteolysis etc. [28]. The overall charge of the bacterial cell at physiological pH is negative due to the dissociation of excess carboxylic groups at the cell surface [29]. Since NiO has a positive surface charge, so, they became electrostatically bound to the negative cell surface,

TABLE 1. Zone of inhibition for tetracycline, gentamicin, bulk NiO and NiO nanoparticles (NPs) against *S. aureus* and *E. coli* at different concentrations

Conc. ( $\mu\text{g/ml}$ )	Zone of inhibition (mm) against <i>S. aureus</i>				Zone of inhibition (mm) against <i>E. coli</i>			
	Tetracycline	Gentamicin	Bulk NiO	NiO NPs	Tetracycline	Gentamicin	Bulk NiO	NiO NPs
128.0	22.17	15.67	18.08	25.17	17.58	12.33	15.33	22.08
64.0	19.17	13.75	17.00	22.83	16.00	11.17	14.25	21.33
32.0	16.50	12.25	16.25	21.00	14.00	10.50	13.42	20.92
16.0	13.08	10.50	15.17	19.50	11.83	8.75	12.42	18.58
8.0	11.50	9.00	14.42	18.50	9.50	7.75	11.17	17.50
4.0	8.83	7.42	12.00	17.67	8.00	7.08	10.67	16.33
2.0	7.50	7.08	11.25	16.00	7.25	7.00	10.25	14.08
1.0	7.25	–	10.58	13.50	7.08	–	9.42	12.58
0.5	–	–	9.50	13.17	–	–	8.67	12.08
0.25	–	–	9.00	11.00	–	–	8.25	10.00
0.125	–	–	8.50	9.83	–	–	7.58	8.83

which hinder the cellular activity. Penetration of NiO into the cell and its toxicity made the cell inactive and dead followed by lysis [30]. The penetration of NiO nanoparticles in bacteria through cell membrane leads to generation of reactive oxygen species (ROS), which leads to damage of cell components [31, 32]. The antibacterial potential of the nanoparticles is mainly governed by the size, shape, dosage, stability, morphology, and treatment time [33]. In the present study, the smaller size of NiO nanoparticles was thought to be responsible for better antibacterial actions than its bulk form, as smaller size usually enhanced by the dispersibility and penetration into the intracellular matrix and interference with intracellular  $\text{Ca}^{2+}$  absorption leading to cell damage [22]. Attachment of the nickel ions released from the NiO nanoparticles to the cell membrane of the microbes initiate electrostatic interactions, wherein it interferes with cellular physiology leading to their disruption [34]. Further, these damaged cell membranes are more susceptible to further interactions, which lead to more penetration of NiO nanoparticles and leakage of the intracellular organelles [22]. These sequential steps augment the antimicrobial action of the nanoparticles. Additionally, the interactions of penetrated NiO nanoparticles generate the ROS, which has a significant tendency in interfering the microbial electron transport chain, damaging DNA by breaking the phosphate and hydrogen bonds, denaturing the protein by alteration of the tertiary structure, and damaging the mitochondria by oxidative stress [22, 35]. The soluble nickel compounds attack the bacterial cell membrane from outside, whereas nanoparticles (metal/metal oxide) easily get entry through bacterial cell membrane and leading to the death of bacterial cell [36–38]. The schematically possible hypothetical mechanisms of NiO nanoparticles for the antibacterial actions are shown in Fig. 3. So, the direct contact of NiO nanoparticles may be responsible for damaging the cell membrane followed by cell lysis in present study. It has been observed that metal oxide nanoparticles do not generate bacterial resistance and are safe potential antimicrobial alternatives for clinical applications [39, 40]. Most of the nanoparticles also did not inactivate or lose their properties when exposed to sterilization by different ways, like high temperature, gamma irradiation or plasma treatment etc. [41]. The various advantages and unique properties of nanoparticles have led to the incorporation of nano forms of oxides of silver, copper, titanium, zinc, iron etc. into the medical field has emerged as an alternative to new antimicrobial agents in recent years [42, 43]. Further, NiO nanoparticles in comparison to its bulk form undergo marked transformations, which induce significant changes in their structural and physico-chemical properties, which in turn affects its better action.

Consideration of an antimicrobial agent as better than others can be done after the comparison its potential with some standard antimicrobials and its other forms. The calculation of activity index (AI) by using the values of zone of inhibition is considered one of the standard approaches for the comparison of antibacterial potentials of different compounds [44]. The testing drug/compound will be considered better against particular bacteria if it possess value of AI more than one. In the present investigation, the AI for NiO nanoparticles with respect to tetracycline, gentamicin and bulk NiO against *S. aureus* and *E. coli* was calculated at various concentrations, and the values are presented in Table 2. The AI of NiO nanoparticles with respect to tetracycline, gentamicin and bulk NiO against *S. aureus* and *E. coli* were found higher than 1 at all compared concentrations. These values also revealed that the AI of NiO nanoparticles against *S. aureus* and *E. coli* was higher for gentamicin in comparison to tetracycline and bulk NiO at the same concentration. Another method for the comparative studies of different antimicrobials is calculation of

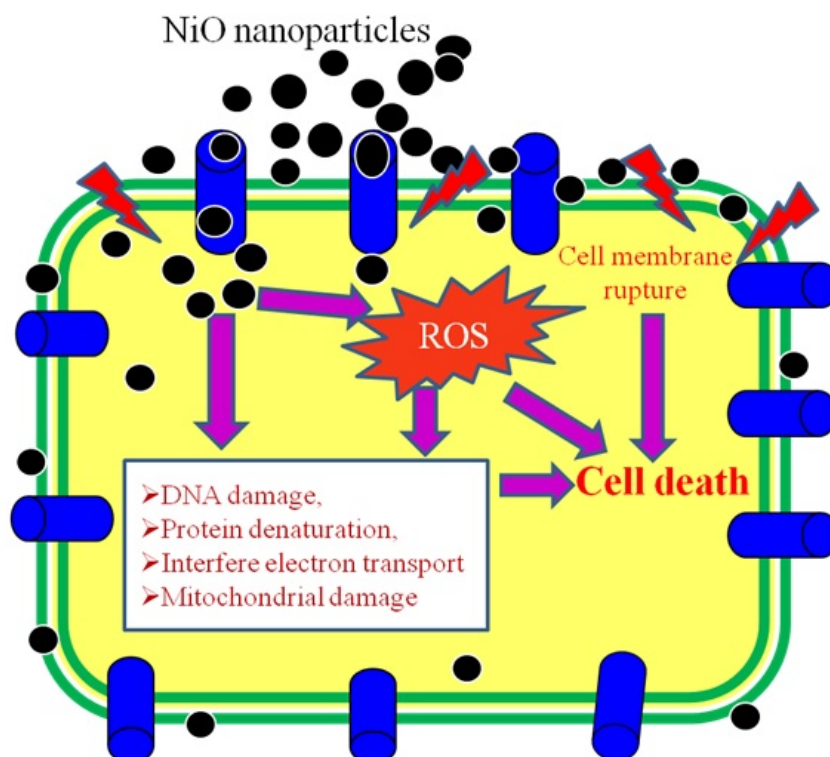


FIG. 3. The schematically possible hypothetical mechanisms of action of NiO nanoparticles for antibacterial activity

TABLE 2. Activity index of NiO nanoparticles with respect to tetracycline, gentamicin and bulk NiO against *S. aureus* and *E. coli* at different concentrations

Conc. ( $\mu\text{g/ml}$ )	Activity Index w.r.t. Tetracycline		Activity Index w.r.t. Gentamicin		Activity Index w.r.t. bulk NiO	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
128.0	1.14	1.26	1.61	1.79	1.39	1.44
64.0	1.19	1.33	1.66	1.91	1.34	1.50
32.0	1.27	1.49	1.71	1.99	1.29	1.56
16.0	1.49	1.57	1.86	2.12	1.29	1.50
8.0	1.61	1.84	2.06	2.26	1.28	1.57
4.0	2.00	2.04	2.38	2.31	1.47	1.53
2.0	2.13	1.94	2.26	2.01	1.42	1.37
1.0	1.86	1.78	–	–	1.28	1.34
0.5	–	–	–	–	1.39	1.39
0.25	–	–	–	–	1.22	1.21
0.125	–	–	–	–	1.16	1.16

fold increase (%). This method represents the relative effect of tested compound in contrast to any standard antibiotic/drug/other compound and the positive value represents the better actions of tested compound. Conversely, the negative value of fold increase indicates that the tested compound is less effective against particular bacteria than standard antibiotic/drug/other compound at the same concentration. In our study, the values of fold increase (%) for NiO nanoparticles with respect to tetracycline, gentamicin and bulk NiO against *S. aureus* and *E. coli* at different concentrations are presented in Table 3. The positive values of fold increase of NiO nanoparticles w.r.t. tetracycline, gentamicin and bulk NiO against *S. aureus* and *E. coli* were evident at all the concentrations in present study (Table 3).

It is well known phrase in biomedical sciences that the prevention is better than cure, so applying/coating of NiO nanoparticles on large active surface area, on cellulose bandages, uniforms, bed linen, medical equipment etc. may be useful preventive measures to control bacterial/microbial infections and will make a considerable input in reducing bacterial contamination, mortality, treatment of costs etc. In recent years, applications of nanotechnology in the biomedical field have given many beneficial results. In the biomedical field, various devices coated with antimicrobial nanomaterials such as heart valves, catheters, and dental implants have been used and in these devices there is delay or inhibition in the adhesion and growth of bacteria such as *Streptococcus mutans*, *S. epidermis*, and *E. coli*. [45]. The application of nanoparticles of different metals and their oxides like zinc, silver, copper or titanium, etc. has increased because of their bactericidal activity against both Gram-positive and Gram-negative bacterial strains. Silver nanoparticles are commonly and widely employed for different purposes like bactericides in catheters, burn wound care, and in the dental practice [46]. But, there are some issues related to the biological safety of silver nanoparticles and pigmentation effect of silver nanoparticles on teeth [47,48]. NiO is cheaper than silver and NiO nanoparticles may replace the silver nanoparticles in the coming years in various application. Additionally, metals oxide nanoparticles are considered better antimicrobial pharmaceuticals due to their durability, high stability, and low mammalian cell toxicity in comparison to organic nanoparticles [49]. The promising antimicrobial actions shown by different metals oxide nanoparticles, including NiO nanoparticles, could have a several therapeutic applications in biomedical field and their use can also be extended to food industry, water purification, textile industry, paint industry, sewage treatments, etc.

TABLE 3. Fold increase (%) of NiO nanoparticles with respect to (w.r.t.) tetracycline, gentamicin and bulk NiO against *S. aureus* and *E. coli* at different concentrations

Conc. ( $\mu\text{g/ml}$ )	Fold increase (%) w.r.t. Tetracycline		Fold increase (%) w.r.t. Gentamicin		Fold increase (%) w.r.t. bulk NiO	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
	128.0	13.53	25.59	60.64	79.05	39.17
64.0	19.13	33.33	66.06	91.04	34.31	49.71
32.0	27.27	49.40	71.43	99.21	29.23	55.90
16.0	49.04	57.04	85.71	112.38	28.57	49.66
8.0	60.87	84.21	105.56	125.81	28.32	56.72
4.0	100.00	104.17	138.20	130.59	47.22	53.13
2.0	113.33	94.25	125.88	101.19	42.22	37.40
1.0	86.21	77.65	–	–	27.56	33.63
0.5	–	–	–	–	38.60	39.42
0.25	–	–	–	–	22.22	21.21
0.125	–	–	–	–	15.69	16.48

#### 4. Conclusions

In conclusion, the NiO nanoparticles were better antibacterial agents than their bulk form and they also seemed better than the standard antibiotics tetracycline and gentamicin, particularly at low concentrations, against both gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria. In future, the strong antibacterial potentials of NiO nanoparticles may be extended its applications in different fields.

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