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Research Article

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Synergistic antibacterial activity of zinc oxide nanoparticles with antibiotics against the human pathogenic bacteria

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ABSTRACT

To study synergistic antibacterial activity of zinc oxide nanoparticles incorporated anti bacterial antibiotics against human pathogenic bacteria. Zinc oxide nanoparticles were synthesized by wet method and the synthesized nanoparticles were incorporated ofloxacin, norfloxacin and cephalexin. Anti-bacterial activity was studied against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa adopting agar diffusion assay and biofilm inhibition assay. Zinc oxide nanoparticles were synthesized by wet method showed nanoaggregrates with the size range of 52-75nm.Synthesized nanoparticles with respective antibiotics showed improved anti bacterial activity and the anti bacterial activity of nano drug conjugate against all the tested pathogenic bacteria reveals increase in zone of inhibition and biofilm inhibition as dose dependent manner. The present study would suggests the possible utilization of zinc oxide nanoparticles incorporated antibiotics as an effective anti bacterial agent against pathogenic bacteria.

Keywords: Zinc oxide nnaoparticles, antibiotics, anti-bacterial, biofilm

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INTRODUCTION

With growth in world population and the spread of disease, the number of antibiotic resistant microorganisms is rising along with the occurrence of infections from these microorganisms. With this increase in health awareness, many people focused their attention on educating and protecting themselves against harmful pathogens. Nanoscale science and technology have emerged over the past decade as the forefront of science and technologies. The interesting fields of study that create this domain of science and engineering perfectly typify the rapid, multidisciplinary advancement of contemporary science and technology. Inorganic materials such as metal and metal oxides have attracted lots of attention over the past decade due to their ability to withstand harsh process conditions [1,2]. Of the inorganic materials, metal oxides such as TiO_2, ZnO, MgO and CaO are of particular interest as they are not only stable under harsh process conditions but also generally regarded as safe materials to human beings and animals [3,4]. The use of nanoparticles of silver and zinc oxide has been seen as a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles. Taken together, this compound as a highly safe compound may be considered for combination therapy against pathogenic microorganism due to its potential synergistic effect with important antibiotics [5].

ZnO nano-particles have some advantages, compared to silver nano-particle, such as lower cost, white appearance [6] and UV-blocking property [7]. ZnO powders can absorb infra-red light and infra-red electromagnetic wave with 5–16.68 dB in the range of 2.45–18GHz [8]. ZnO is also used to reinforce polymeric nano-composites [9]. They also appeared for enhancement wear resistant phase and anti-sliding phase in composites as a consequence of their high elastic modulus and strength [10]. In the present study, synergestic effect of zinc oxide nanoparticles with anti bacterial antibiotics against human pathogenic bacteria has been studied.

EXPERIMENTAL SECTION

Zinc oxide nanoparticles were prepared by wet chemical method [11] using zinc sulphate and sodium hydroxide as precursors and soluble starch as stabilizing agent. Different concentrations of soluble starch (0.1%, 0.5% and 1.0%) were dissolved in 500 ml of distilled water by using microwave oven. Zinc sulphate, 17.945g (0.1 M) was added in the above solution. Then the solution was kept under constant stirring using magnetic stirrer to completely dissolve the zinc sulphate. After complete dissolution of zinc sulphate, 0.2 M of sodium hydroxide solution (20 ml was used in our study) was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 hours after complete addition of sodium hydroxide. After the completion of reaction, the solution was allowed to settle for overnight and the supernatant solution was the discarded carefully. The remaining solution was centrifuged at 10,000 X g for 10 mins and the supernatant was discarded. Thus obtained nanoparticles were washed three times using distilled water. Washing was carried out to remove the discarded. Thus obtained nanoparticles were washed three times using distilled water. Washing, the nanoparticles were dried at 80^{o}C for overnight. During drying, complete conversion of zinc hydroxide into zinc oxide takes place.

Antibacterial activity

Preparation of nanosuspension

Synthesized nanoparticles was suspended in 1 ml of dilute sodium hydroxide solution (0.1N) in 1.5 ml sterile eppendorf tubes, kept in cyclomixer for the complete dispersion. Working solution was prepared from the stock as 10,50 and 100 **µg** final concentration and used for further study.

Well diffusion assay

The anti bacterial activity of nanoparticles was studied against *Pseudomonas* a*euroginosa* (ATCC 10145), *Escherichia coli* (ATCC 21210 and *Staphylococcus aureus* (ATCC) adopting well diffusion assay[12]. Bacterial strains were obtained from American type culture collection (ATCC) and maintained on tryptic soy agar (TSA) slants. A loopful of slant culture was inoculated into tryptic soy broth and incubated at 37ºC for 12 hours under shaking condition in orbital shaker (Remi, Mumbai, India). The respective broth culture was uniformly spread with sterile cotton swabs on sterile Mueller Hinton (MH)Agar Media (Hi-media, India). The wells were made using cork borer and the respective concentration of nanoparticles suspension was loaded into the wells. The plates were incubated at 37ºC for 24 hrs.

Evaluation of synergestic anti bacterial activity

Norfloxacin, ofloxacin and cephalexin were selected in the present study. All the antibiotics were obtained from Hi media,Mumbai,India.0.1mg/ml of respective antibiotics was mixed with respective concentration of zinc oxide nanoparticles ,the mixture was kept under stirring for three hours at room temperature. After stirring, the suspension was lyophilized and used for further studies. Lyophilized nano drug conjugate with different concentration (10,50 and 100 µg was used for anti bacterial activity against tested organisms as described earlier.

Biofilm inhibition study

Inocula preparation

Respective bacterial culture was inoculated from fresh slopes of TSA into tryptic soy broth (TSB) and incubated with shaking at 37 °C for 24 hours. Cells were collected by centrifugation and the collected cell debris washed twice in PBS and suspended to OD_{520} prior to use in biofilm experiments [13].

Biofilm inhibition assay

Spectrophotometric microtitre plate assay was used to study biofilm inhibition study.100 µL of respective bacterial cell suspension and the respective concentration of nanoparticles and antibiotics incorporated zinc oxide nanoparticles was added into the wells of a 96-well PVC microtiter plate. The microtiter plates were covered and sealed before incubation under stationary conditions at 37 °C for 24 hours. After the incubation time, the content was discarded and the plates thoroughly washed with water. 100 µl of 0.1% aqueous solution of crystal violet was added and incubated at room temperature for 30 minutes followed by washing with water the remaining stain was solubilized with 200 µL of 95% ethanol. Biofilm inhibition was studied by determination of the absorbance of the ethanol solubilised mixture at 540 nm in an UV spectrophotometer. Control (without bacteria only crystal violet), three replicates were maintained for each treatment [14].

RESULTS AND DISCUSSION

The discovery of antibiotics in the 20th Century marked a watershed in the treatment of infections. The ability to treat the serious infections of the pre-antibiotic era stimulated advances in medical fields and enlarged the scope of medical care. However, while a drastic change has taken place in the causes of fatal infections, they are still a major

cause of death the world over [15].While demographic changes and drug access issues are important reasons in the developed and developing worlds, respectively, "relentless and Dizzying Rise of Antimicrobial Resistance" has contributed in a large measure to the persistence of infections as a major cause of morbidity and mortality [16]. The rapid emergence of resistance to antibiotics amongst pathogens generates visions of the 'potential post-antibiotic era threatening present and future medical advances. In the present study, synergestic anti bacterial activity of zinc oxide nnaoparticles with antibiotics against human pathogenic bacteria was studied.ZnO_2 nanoparticles were synthesized by the chemical reduction of zinc sulphate with sodium hydroxide using starch as the stabilizer. Among the different concentration of starch used,1 % starch yield zinc oxide particles with nano scale which was confirmed by scanning electron microscopy with size range of 98.2-99.2 nm (Figure 1).

Table 1.Zone of inhibition (mm) of free ZnO nanoparticles against tested pathogenic bacteria

S. No.	Tested Bacterium	Zone of inhibition (cm)		
		10 µg	50 µg	100 µg
1	*Staphylococcus aureus*	1.6	1.8	1.9
2	*Escherichia coli*	1.4	3.0	3.1
3	*Pseudomonas aeruginosa*	1.9	2.3	2.7

Table 2: Zone of inhibition (mm) of ZnO nanoparticle with Ofloxacin, Norfloxacin, Cephalexin

S. No.	Tested Bacterium	Treatment	Zone of inhibition (cm)		
			10 µg	50 µg	100 µg
1	*Staphylococcus aureus*	Ofloxacin	1.4	2.5	3.1
		Norfloxacin	3.1	4.4	5.0
		Cephalexin	2.9	3.7	3.8
2	*Escherichia coli*	Ofloxacin	3.7	4.2	5.5
		Norfloxacin	3.3	3.8	4.0
		Cephalexin	3.7	4.0	4.2
3	*Pseudomonas aeruginosa*	Ofloxacin	3.4	3.7	4.3
		Norfloxacin	4.0	4.2	4.5
		Cephalexin	3.1	3.7	3.9

Table 3: Biofilm inhibition (%) of pathogenic bacteria with ZnO nanoparticle

S. No.	Tested Bacterium		Bio film inhibition (%) (O.D at 570 nm)			
			10 µg	25 µg	50 µg	100 µg
1	*Staphylococcus aureus*	Control	0.00	0.00	0.00	0.00
		Treatment	20.93	23.39	26.09	29.01
2	*Escherichia coli*	Control	0.00	0.00	0.00	0.00
		Treatment	15.22	20.56	26.09	32.55
3	*Pseudomonas aeruginosa*	Control	0.00	0.00	0.00	0.00
		Treatment	14.23	18.16	25.57	30.98

Table 4: Bio film inhibition (%) of pathogenic bacteria with ZnO nanoparticle and antibiotics

S. No.	Tested Bacterium	Treatment	Bio film inhibition (%) (O.D at 570 nm)			
			10 µg	25 µg	50 µg	100 µg
1	*Staphylococcus aureus*	NP+Ofloxacin	47.3	59.2	58.4	66.9
		NP+Norfloxacin	69.7	79.3	86.6	95.5
		NP+Cephalexin	16.47	25.95	35.80	49.89
2	*Escherichia coli*	NP+Ofloxacin	27.74	20.00	19.80	14.26
		NP+Norfloxacin	61.66	53.33	50.68	40.13
		NP+Cephalexin	15.00	9.03	8.44	7.99
3	*Pseudomonas aeruginosa*	NP+Ofloxacin	93.5	77.4	77.4	74.2
		NP+Norfloxacin	62.6	61.3	52.9	48.9
		NP+Cephalexin	25.44	25.26	24.40	20.27

Anti bacterial activity of free zinc oxide reveals free nanoparticles inhibited all the tested bacteria. In *S. aureus*, the zone of inhibition was found to be 16, 18, 19 mm at 10, 50, 100 µg concentrations (Table 1). 14, 30, 31 mm and 19,21,23 mm of zone of inhibition was recorded in *E. coli* and *P. aeruginosa*. The antibacterial activity of ZnO has been found to be due to a reaction of the ZnO surface with water and production of elevated levels of reactive oxygen species, namely hydroxyl radicals and in turn induces oxidative stress. Similarly, an exposure of bacteria to the small ZnO nanoparticles results in an increased cellular internalization of the nanoparticles and bacterial cell damage[17].

Figure: 1 shows SEM image of free zinc oxide nanoparticles

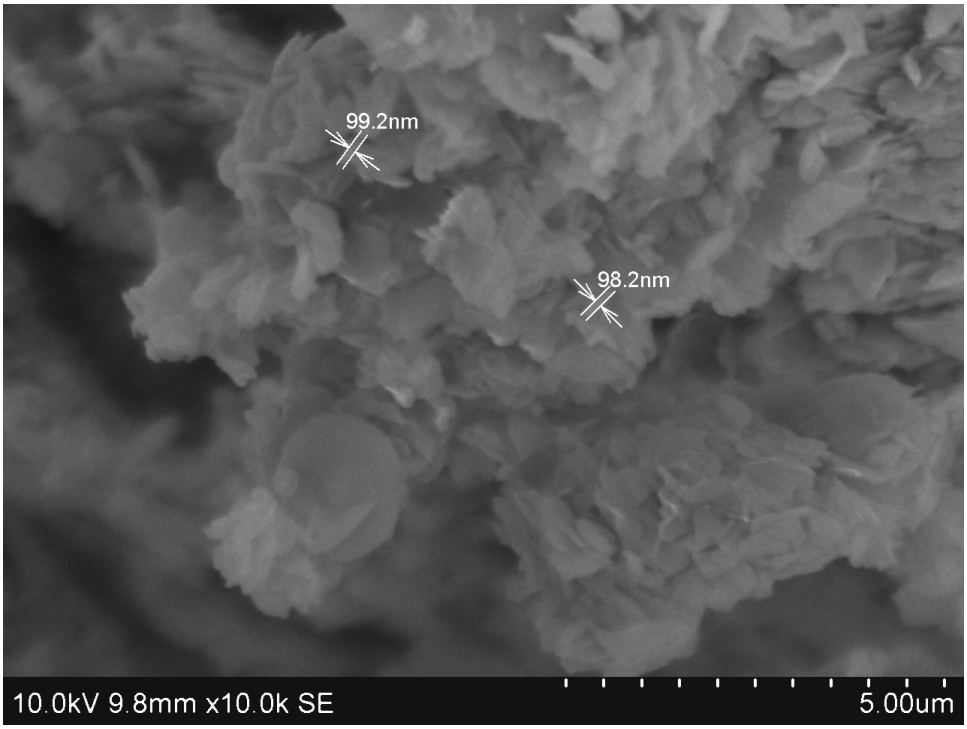

Figure 2a.Zone of inhibition of nano drug conjugate against *S. aureus*

Figure 2b.Zone of inhibition of nano drug conjugate against *E. coli*

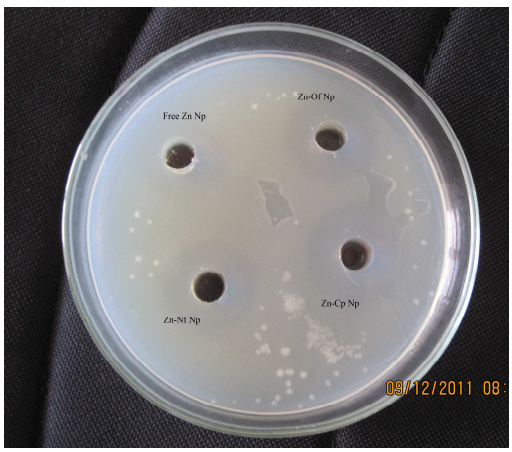

Figure 2c.Zone of inhibition of nano drug conjugate against *P.aeruginosa*

The ZnO nanoparticles with all the antibiotics tested showed maximum anti bacterial activity against all the tested pathogenic bacteria, which clearly reveals the synergistic activity of antibiotic with ZnO nanoparticles (Figure 2,a,b,c).In *S. aureus*, the zone of inhibition of ZnO nanoparticles with ofloxacin was 14, 25, 31mm at 10, 50, 100 µg concentrations (Figure 2a). In *E. coli*, the zone of inhibition of ZnO nanoparticles with ofloxacin is 37, 42, 55 mm at 10, 50, 100 µg concentrations. In *P. aeruginosa*, the zone of inhibition of ZnO nanoparticles with ofloxacin is 34, 37, 43 mm at 10, 50, 100 µg concentrations (Table2).Similar enhanced anti bacterial activity was recorded in norfloxacin. In *S. aureus*, the zone of inhibition of ZnO nanoparticles with norfloxacin is 31, 44, 50 mm at 10, 50, 100 µg concentrations. In *E. coli*, the zone of inhibition of ZnO nanoparticles with norfloxacin is 33, 38, 40 mm at 10, 50, 100 µg concentrations In *P. aeruginosa*, the zone of inhibition of ZnO nanoparticles with norfloxacin is 40, 42, 45 mm at 10, 50, 100 µg concentrations. Cephalexin also revealed similar anti bacterial activity against all the tested bacteria. In the present study, nanoparticles incorporated with the antibiotics showed enhanced activity against all the tested bacteria. Small size and corresponding large specific surface area of small nanometer-scale ZnO particles with the respective antibiotics impose several effects that shows their enhanced antibacterial action. Zinc nanoparticles have been shown to have enhanced good safety profile and no toxicity observed when taken at different nano sizes of the zinc particles [18].Taken together, this compound as a highly safe compound may be considered for combination therapy against pathogenic bacteria due to its potential synergistic effect with important antibiotics.

Biofilm study shows distinct inhibition of biofilm on the all the tested bacteria by the both the free and antibiotics incorporated zinc oxide nanoparticles. Anti biofilm effect of metallic nanoparticles was reported [19,20]. Free zinc oxide nanoparticles recorded inhibition against all the tested bacteria(Table 3). Biofilm inhibition study with drug nano conjugate reveals maximum inhibition was recorded in all the antibiotics incorporated nanoparticles (Table 4).

CONCLUSION

The present study would suggests the possible utilization of zinc oxide nanoparticles incorporated antibiotics to prevent the fatal infections caused by the pathogenic bacteria Further study will helpful to formulate nano drug conjugate as an anti-microbial agent in a large scale level through standardized regulatory conditions.

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