Effect of Metal Hydroxide Nanoparticles against different isolated Bacterial Species

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***Abstract*— A nanoparticle (or nano-powder or nano-crystal or nano-cluster or nano-material) is a microscopic particle with at least 1D less than 100nm.A variety of metal hydroxide nanoparticles have shown success for use as vehicles for drug delivery, targeted gene delivery, and tumour imaging. This is typically because nanoparticles have a greater surface area per weight than larger particles, which cause them to be more reactive to some other molecules. In the present project work preparation of metal hydroxide nanoparticles and toxicological study of it on isolated bacterial species was done. In this study, Zinc Hydroxide NPs, Iron Hydroxide NPs and Magnesium Hydroxide NPs were rapidly synthesized from Zinc Nitrate, Ferric Chloride and Magnesium Chloride respectively. And these prepared different metal hydroxide nanoparticles were tested for its antibacterial activity on isolated bacterial species Micrococcus Leteus and Staphylococcus Aureus, by using agar well diffusion method. The zone of inhibition was observed and recorded to determine its antibacterial activity. The inhibition of bacterial growth was tested for prepared nanoparticles on diluting it, by MIC test. The term "nanoparticles" means only solitaire nanoparticles. The maximum growth was inhibited by ferric hydroxide nanoparticles and minimum growth of bacteria inhibited by Zinc hydroxide and Magnesium Hydroxide.**

***Keywords*— Metal Hydroxide Nanoparticles, Ferric/Iron Hydroxide NPs, Zinc Hydroxide NPs, Magnesium Hydroxide NPs, Micrococcus luteus, Staphylococcus aureus**

I. INTRODUCTION

Nanotechnology represents a new and enabling platform that promises to provide a broad range of novel uses and improved technologies for biological and biomedical applications. One of the reasons behind the intense interest is that nanotechnology permits the controlled synthesis of materials where at least one dimension of the structure is less than 100 nm. This ultra-small size is comparable to naturally occurring proteins and biomolecules in the cell, and is notably smaller than the typical diameter (~7 μm) of many human cells. The reduction of materials to the nanoscale can frequently alter their electrical, magnetic, structural, morphological, and chemical properties enabling them to interact in unique ways with cell biomolecules and enable their physical transport into the interior structures of cells. Nanoscale particles typically possess a larger percentage of atoms at the material's surface, which can lead to increased surface reactivity, and can maximize their ability to be loaded with therapeutic agents to deliver them to target cells. By appropriate engineering design these nanomaterials can acquire the ability to selectively target particular types of cells or to pass through physiological barriers and penetrate deep into tumor sites (Rasmussen et al., 2010).

In recent years, semiconductor zinc oxide (ZnO) has gained momentum due to their unique properties such as electronic, structural and thermal. It has been used considerably for its important applications in different areas viz. catalysts, sensors, optoelectron, highly functional and effective photoelectron devices. ZnO nanostructures have a great advantage to apply in medical and pharmaceutical applications due to their large surface area and high catalytic activity. The ZnO is widely used in baby powder, calamine cream, anti-dandruff shampoos, and antiseptic ointments as a potential antimicrobial agent (Singh et al., 2014).

Considerable antibacterial efficiency was observed for ZnO, MgO, and Ti2O and attributed to the ability of these nanoparticles to: (i) easily bind and damage the bacterial membrane, (ii) penetrate the cell and bind to a specific target, and (iii) generate reactive oxygen species (ROS) on their surfaces that in turn provoke an enhancement of the intracellular oxidative stress. Compared to the organic molecules at a larger scale, metal oxide nanoparticles reveal enhanced temperature stability and may attack bacteria via multiple molecular mechanisms. As a consequence, bacteria are unlikely to develop resistance against them since a series of mutations of the microorganism would be necessary in order to become resistant to the treatment (Vidic et al., 2013).

ZnO NPs are toxic to antibiotic (methicillin)-resistant bacteria such as Streptococcus agalactiae (+) and S. aureus. These NPs are able to disorganize and damage the cell membrane and increase the permeability, which leads to cell death. The polyvinyl alcohol (PVA)-coated ZnO NPs are able to internalize the bacteria and induce oxidative stress. The toxicity of ZnO NPs is concentration-dependent and these NPs are mildly toxic at low concentration (Wang et al., 2006).

The interaction of nanoparticles with the organism has to be studied at cellular and molecular levels, in lungs as well as in those secondary target organs which receive sufficiently high doses. Microscopic analyses of such organs from animal inhalation experiments may provide more detailed information about possible pathways responsible for (adverse) effects. It will be important to know which tissues, cells and/or

subcellular compartments nanoparticles interact with and what particle properties are crucial for these interactions. Unrestricted crossing of the cellular membranes by nanoparticles facilitates not only their translocation into basically any organ but also into cells and subcellular compartment (Geiser & Kreyling, 2010).

Non-essential metals, such as silver, can be toxic to bacteria, having biocidal activities at exceptionally low concentrations, while essential metals, such as copper, can also be lethal above some threshold despite their relevance in the biochemistry of organisms. Because of this biocidal activity, metals have been widely used for centuries as antimicrobial agents in agriculture, healthcare, and industry in general. Metal, oxide, or salt compounds based on copper and silver are among the most widely applied antimicrobial agents in this context (Palza, 2015).

Nanoparticles once released into the environment organisms are likely to be exposed in different ways. Human toxicity studies, with a main focus on workers environments, not only primarily consider dispersion and uptake from air, but also direct uptake through ingestion, dermal exposure and, in some special cases related to medical use, injections. Environmental issues also concern air, as particulate matter dispersed in air will fall out by gravity once they are condensed or aggregated and thus reach a certain size. Both in this and other scenarios for spreading nanoparticles, water may serve as a transport medium and a temporary reservoir for nanoparticles. Yet, the ultimate recipients for any non-volatile compound or particle spreading in the environment will be sediments and soils (Joner et al., 2008).

II. Materials & Methodology

Sample Collection: Soil sample were collected to isolate bacterial species.

Isolation of bacteria from soil sample by Serial dilution method: The concentration of bacteria in sample was decreased by using serial dilution method. By using serial dilution method we can obtain optimum amount of bacteria that can easily grow on medium and also can easily identified.

Pure Culture Preparation of bacterial species by Streak plate method: Used Nutrient agar media i.e. suitable for growth of bacterial species, incubated at 37oC for 24 Hrs.

Spreading plate method of soil sample on NAM- Soil sample was spread over solidifies NAM to obtain multiple growth colonies of bacteria, incubated at 37oC for 24 Hrs.

Identify the bacterial species: For identification of microorganisms, firstly we proceed by Gram's Staining and after that we precede some biochemical tests in laboratory according Bergey's manual.

Preparation of Metal Hydroxide Nanoparticles-
Preparation of Zinc Hydroxide Zn (OH)2 Nanoparticles:
Prepare a 1M solution of Zinc Nitrate (ZnNO3) and 1M solution of sodium hydroxide (NaOH). Keep the zinc nitrate solution on heating mantle or magnetic stirrer with hot plate. When solution starts boiling, start adding 1M sodium hydroxide solution dropwise using micro-pipette or dropper. Add the sodium hydroxide solution till entire amount is added to it. After complete addition of sodium hydroxide solution, heat the mixture for 1 hour at 200˚C with intermittent mixing.

Preparation of Iron Hydroxide [Fe(OH)2]3 Nanoparticles: Prepare a 1M solution of Ferric chloride (FeCl2) and 1M solution of sodium hydroxide (NaOH). Keep the Ferric chloride solution on heating mantle or magnetic stirrer with hot plate. When solution starts boiling, start adding 1M sodium hydroxide solution dropwise. Add the sodium hydroxide solution till entire amount is added to it. After complete addition of sodium hydroxide solution, heat the mixture for ≈2 hour at 200 ˚C with intermittent mixing.

Preparation of Magnesium Hydroxide Mg(OH)2 Nanoparticles: Prepare a 1M solution of Magnesium chloride (MgCl2.6H2O) and 1M solution of sodium hydroxide (NaOH). Keep the magnesium chloride solution on heating mantle or magnetic stirrer with hot plate. When solution starts boiling, start adding 1M sodium hydroxide solution dropwise. After complete addition of sodium hydroxide solution, heat the mixture for 2 hour at 200 ˚C with intermittent mixing.

III. Result & Discussion

Isolation of Bacteria spreading plate method-
Spreading of serially diluted soil sample occurred on NA Media, to grow the bacterial species.

Isolation of Bacteria streaking plate method-
Streaking of bacterial culture occurred to obtain the colonies of single bacteria, on NA media

Fig.:1- Streaking of bacterial colony (A) BS-1 (B) BS-2

Morphological Study of Bacteria by Gram Staining- Two bacterial species was isolated from different soil sample named as BS-1 & BS-2.The bacterial species was stained with Gram's stain in order to study its morphology. The bacterial species BS-1 & bacterial species BS-2 both was found to be

Gram's positive, cocci. The probability of Bacterial spp. may be *E. coli, Corynebacterium, Bacillus, Neisseria, Pseudomonas, Micrococcus, Staphylococcus, Streptococcus, Enterococcus, Lactococcus and Aerococcus spp.*

According to result of various biochemical tests performed by bacterial species BS-1 and BS-2, it was determined that, BS-1 was *Micrococcus leuteus* and BS-2 was *Staphylococcus aureus.*

TABLE I
MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL SPECIES BY DIFFERENT STAINING TECHNIQUES

S.No.	Test	Bacteria BS-1	Bacteria BS-2	Shape
1	Gram's staining	Gram Positive Bacteria	Gram Positive Bacteria	BS-1:Cocci
				BS-2:Cocci
2	Acid fast staining	Non-acid fast	Non-acid fast	BS-1: Cocci
				BS-2: Cocci
3	Endospore Staining	Non-endospore	Non-endospore	BS-1: Cocci
				BS-2: Cocci

TABLE 2
BIOCHEMICAL CHARACTERISTICS OF BACTERIAL SPECIES

S.No.	Biochemical Test		Result	
			BS-1	BS-2
1	Sugar Fermentation Test	Sucrose	Negative	Positive
		Glucose	Negative	Positive
		Mannitol	Negative	Positive
2	MR Test		Positive	Positive
3	VP Test		Negative	Negative
4	Indole Test		Positive	Positive
5	Catalase Test		Negative	Positive
6	Citrate Utilization Test		Negative	Negative
7	Urease Test		Positive	Positive
8	Casein Hydrolysis Test		Positive	Positive
9	Starch Hydrolysis Test		Negative	Negative

Antibacterial Activity Test of prepared nanoparticles on different isolated bacteria:

Antibacterial activity test of Zinc Hydroxide Nanoparticle on isolated bacterial species-The zone of inhibition on growth of bacteria on BS-1, by antibiotic solution (control) was 45mm and by no antimicrobial activity was observed for NPs against the bacteria. And on BS-2, by antibiotic solution (control) was 30mm & by no activity was seen in the case of prepared NPs.

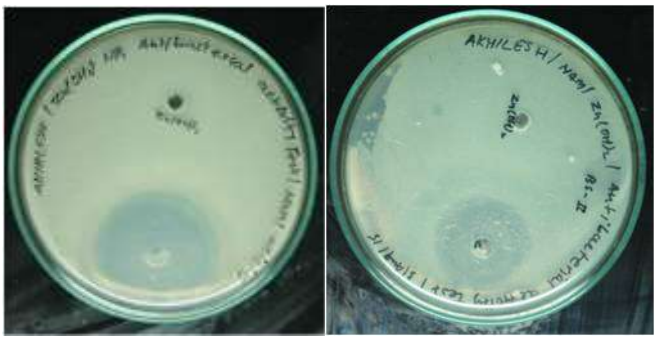

Fig.:2- The inhibited zones of bacterial growth shown by Zinc Hydroxide NPs

Antibacterial activity test of Iron Hydroxide Nanoparticle on isolated bacterial species-

The zone of inhibition of growth of bacteria on BS-1, by antibiotic solution (control) was 40mm and by NPs was 21mm. And on BS-2, by antibiotic solution (control) was 34mm & by NPs was 28mm.

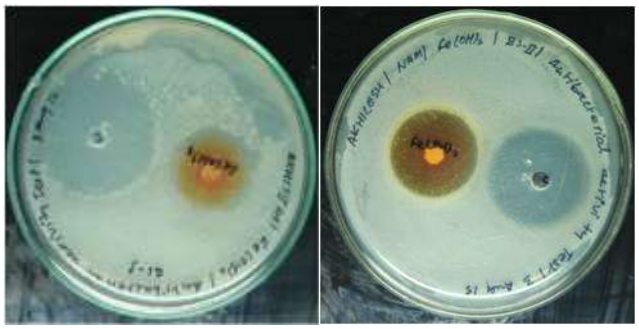

Fig.:3- The inhibited zones of bacterial growth shown by Iron Hydroxide NPs

Antibacterial activity test of Magnesium Hydroxide Nanoparticle on isolated bacterial species-

The zone of inhibition of growth of bacteria on BS-1, by antibiotic solution (control) was 38mm and by NPs was Nil. And on BS-2, by antibiotic solution (control) was 29mm & by NPs was Nil.

Fig.:4-The inhibited zones of bacterial growth shown by Magnesium Hydroxide NPs

TABLE 3
ANTIBACTERIAL ACTIVITY SHOWN BY PREPARED NPS ON ISOLATED BACTERIAL SPECIES.

S. No.	Nanoparticles	Zone of Inhibition			
		BS-1		BS-2	
		+ve control	NPs	+ve control	NPs
1	Zinc Hydroxide NPs	45mm	0mm	30mm	0mm
2	Iron Hydroxide NPs	40mm	21mm	34mm	28mm
3	Magnesium Hydroxide NPs	38mm	0mm	29mm	0mm

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

Minimum Inhibitory Concentration (MIC)-

Prepared metal hydroxide nanoparticles were analyzed for their antibacterial activity against BS-1 and BS-2. It was observed that microbial growth of different microbes was dependent on metal hydroxide NPs concentration. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of metal hydroxide nanoparticles that inhibited the visible growth of BS-1 and BS-2. It was found that the MIC for metal hydroxide nanoparticles, and the lowest concentration of metal hydroxide nanoparticles that inhibited the visible growth of BS-1 and BS-2. These results indicated that the MIC of metal hydroxide nanoparticles. The lowest concentration of metal hydroxide NPs that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism was recorded as the MIC values. The MIC values of Zinc Hydroxide nanoparticle for BS-1 is 10^{-5} and for BS-2 is 10^{-4}, MIC values of Iron Hydroxide nanoparticle for BS-1 is 10^{-6} and for BS-2 is 10^{-5}, and MIC values of Magnesium Hydroxide nanoparticle for BS-1 is 10^{-5} and for BS-2 is 10^{-4} .

TABLE 4
MIC VALUES OF PREPARED METAL HYDROXIDE NPS FOR ISOLATED BACTERIAL SPECIES.

S. No.	Nanoparticles	MIC Values (Diluted concentration of NPs with NB Media)	
		BS-1	BS-2
1	Zinc Hydroxide	10^{-5}	10^{-4}
2	Iron Hydroxide	10^{-6}	10^{-5}
3	Magnesium Hydroxide	10^{-5}	10^{-4}

Minimum bactericidal concentration (MBC)-

The MBC was the concentration at which the bacteria are completely killed. The antibacterial activities increased as the concentration of metal hydroxide nanoparticles increased. The lowest concentration of nanoparticles, retained completely inhibition of growth of bacteria, recorded as MBC values.

IV. CONCLUSION

The present study demonstrated that, the different soil sample was collected to isolate bacterial species. The soil sample was used to isolate bacteria by using serial dilution and after that pure culture preparation was done. There was two bacterial species isolated from different soil sample, and denoted as BS-1 & BS-2. The isolated bacterial species BS-1 was identified as Micrococcus leutius and BS-2 was identified as Staphylococcus aureus according to Bergey's Manual based on performed biochemical tests.

Different metal hydroxide nanoparticles was prepared, that was Zinc Hydroxide NPs (ZnOH2), Iron Hydroxide NPs [Fe(OH2)3] and Magnesium Hydroxide NPs (MgOH2), using metal nitrates & chloride with sodium hydroxide.

Antibacterial activity of prepared metal hydroxide nanoparticles was tested on Micrococcus Leutius and Staphylococcus Aureus. In antibacterial activity test zone of inhibition was observed. The maximum zone of inhibition was observed on BS-2 (Staphylococcus aureus), 34mm by Ferric/Iron Hydroxide NPs, and on BS-1 (Micrococcus leutius), 28mm by ferric/iron hydroxide NPs. And minimum zone of inhibition was observed on BS-1 and BS-2, 0mm by Zinc Hydroxide and Magnesium Hydroxide NPs.

These prepared metal hydroxide nanoparticles inhibited the growth of bacteria after dilution, and it was tested by using MIC Test. Thus from present study it can be concluded that, these metal hydroxide nanoparticles exhibited remarkable antibacterial activity. These nanoparticles causes the cytotoxicity, thus it can help in destruction of disease causal bacteria.

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