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Research Article

SYNTHESIS OF COPPER OXIDE NANOPARTICLES BY CHEMICAL PRECIPITATION METHOD FOR THE DETERMINATION OF ANTIBACTERIAL EFFICACY AGAINST *STREPTOCOCCUS* SP. AND *STAPHYLOCOCCUS* SP.

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ABSTRACT

Objective: To determine antimicrobial efficacy of copper oxide nanoparticles (CuO NPs) against *Streptococcus sp*. and *Staphylococcus sp*.

Methods: CuO NPs were synthesized using chemical precipitation method. The reducing agent, 0.1 M NaOH, was used along with 100 mM $CuSO_4$ precursor for the synthesis of CuO NPs. The characterization of CuO NPs was done by ultraviolet-visible spectroscopy and scanning electron microscopy (SEM) to study optical and morphological characteristics, correspondingly. The identification of bacterial cultures was done through microscopic and biochemical studies. Antibacterial efficacy of CuO NPs was determined against *Streptococcus* sp. and *Staphylococcus* sp. by qualitative and quantitative methods through anti-well diffusion assay and broth dilution method, respectively.

Results: The absorption spectrum and band gap were found to be at 260 nm and 4.77 eV, respectively. The SEM image of CuO NPs shows cluster of nanostructures having width of individual clusters in the range of 100 nm–500 nm. CuO NPs showed inhibition at a concentration ranging from 60 µg/mL to 1000 µg/mL.

Conclusion: Finally, CuO NPs can be used as effective antibacterial agent against *Streptococcus* sp. and *Staphylococcus* sp. and may have applications in medical microbiology.

Keywords: Chemical precipitation, Copper oxide nanoparticles, Antibacterial activity, *Streptococcus* sp., *Staphylococcus* sp.

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INTRODUCTION

The compounds that locally kill bacteria or slow down their growth exhibit antibacterial activity. These compounds are generally not toxic to the surrounding tissue. Most current antibacterial agents are chemically modified natural compounds [1] such as β-lactams (like penicillins), cephalosporins, or carbapenems. In general, the agents can be classified as either bactericidal, which kill bacteria, or bacteriostatic, slowing down bacterial growth.

These antibacterial agents are vital to fight against infectious diseases. However, overuse of such agents has found ineffective and developed resistance in bacteria. Such resistance is most often based on evolutionary processes taking place during antibiotic therapy and leads to inheritable resistance [2]. Such kind of antibacterial-resistant strains and species are unofficially referred to as superbugs and contribute to the appearance of diseases that were under good control for many years. Thus, due to the fact that bacteria developed resistance against many common antibacterial agents, infectious diseases continue to be one of the greatest health challenges worldwide. For conventional antimicrobial agents, the development of multiple drug resistance is not the only downside but also undesirable side effects. Drug resistance enforces high-dose administration of antibiotics and generates unendurable toxicity.

This has driven the development of alternative strategies to treat bacterial diseases [3]. The development of nanoscale materials has emerged as novel antimicrobial agents. Several classes of antimicrobial nanoparticles (NPs) and nanocarriers for antibiotics delivery have proven their efficiency for treating infectious diseases including antibiotic-resistant ones, *in vitro*, as well as in animal models [4]. NPs offer improved properties to classical organic antibacterial agents for various reasons; one reason lies in their high surface area to volume ratio, resulting in appearance of new mechanical, chemical, electrical, optical, magnetic, electro-optical, and magneto-optical properties of the NPs that are different from their bulk properties [5].

When particles are reduced from a micrometer to a nanometer size, their resultant properties can change considerably. Such characteristics allow them to interact closely with bacterial membranes, rather than the effect being only due to the release of metal ions [6]. In theory, metal NPs could be combined with polymers or coated onto surfaces, which may then have a variety of potential antimicrobial applications. The antimicrobial properties of both silver [7] and copper NPs [8] have been reported in the past and both of these have been coated onto or incorporated into various materials [9]. Copper oxide (CuO)/copper (II) oxide/cupric oxide is a semiconducting compound with a monoclinic structure. CuO is the simplest member of the family of copper compounds and it exhibits a range of potentially useful physical properties such as high-temperature superconductivity, electron correlation effects, and spin dynamics [10,11]. However, the information on the possible antimicrobial activity of nano-CuO is limited. CuO is cheaper than silver and can be easily mixed with polymers. They are relatively stable in terms of both chemical and physical properties. The ionic nanoparticulate metal oxides, such as CuO, can be particularly important antimicrobial agents as they have exceptionally high surface areas and unusual crystal morphologies [12]. The aim of this study is to synthesize and characterize CuO NPs and to further examine its possible antimicrobial properties.

METHODS

Sample collection, isolation, and biochemical characterization

The bacterial isolates were collected from the Department of Microbiology, RGITBT and were further characterized for their physiological and biochemical characteristics. Gram staining, capsule staining, sugar fermentation test, citrate utilization test, catalase test, methyl red test, and blood agar test were performed to characterize bacterial isolates.

Synthesis and characterization of CuO NPs

Synthesis of CuO NPs by chemical precipitation

In typical experimental procedure, 100 mM copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was dissolved in 50 mL of distilled water by continuous stirring on magnetic stirrer at room temperature for 30 min. Simultaneously, 100 mM NaOH was prepared in distilled water and added dropwise into the $CuSO_4$ solution with continuous stirring at room temperature. The resultant precipitate was washed by centrifugation at 6000 rpm for 15 min each with deionized water and 70% ethanol. The final product was dried in hot air oven at 60°C for 24 h, followed by calcination at 400°C for 4 h to obtain powder [13].

Characterization of CuO NPs

The structural and morphological features of resultant CuO NPs were characterized by ultraviolet (UV)-visible spectroscopy and scanning electron microscopy (SEM) for identifying their optical and morphological characteristics.

Determination of antibacterial potential of CuO NPs

Antimicrobial activity of the NPs was checked using the anti-well diffusion agar method [14,15]. Mueller-Hinton agar was used for this assay. Fresh inoculum (100 µl) was spread onto the plates. The plates were refrigerated for 10 min for initial attachment of the microbes. CuO NPs of various dilutions (20, 40, 60, 120, 250, 500, and 1000 µg/ml) were prepared and 20 µl of CuO NPs were added in each well, respectively. The plates were incubated at 37°C for 24 h. The zone of inhibition was measured and susceptibility of the CuO NPs was estimated at different concentrations for the determination of minimum inhibitory concentrations (MICs).

RESULTS

Characterization of isolated bacteria

Six bacterial isolates were maintained on MSA medium and studied for their colony characteristics (Table 1). Gram staining was performed. Two isolates were further studied for the identification on the basis of biochemical characterization (Table 2).

Characterization of CuO NPs

UV-visible spectroscopy (Thermo Scientific UV-10) was used to study an optical property of CuO NPs. Spectroscopic results clearly indicated the production of CuO NPs. Absorption spectrums of CuO NPs are shown in Fig. 1. The maximum absorption of synthesized CuO NPs synthesized by chemical precipitation was found to be at 260 nm and the band gap was calculated to be 4.77 eV. The SEM image of CuO NPs shows a cluster of nanostructures having length and width of few microns. However, the width of individual clusters has found to be in the range of 100 nm–500 nm (Fig. 2).

Antibacterial efficacy of CuO NPs by anti-well diffusion assay

Antibacterial activity of CuO NP performed by anti-well diffusion assay indicated inhibition of both *Streptococcus* sp. and *Staphylococcus* sp. Zone of inhibition was observed at concentrations ranging from 60 µg/mL to 1000 µg/mL (Figure 3). No inhibition was observed at concentrations ranging from10 µg/mL to 50 µg/mL. MIC was observed at 60 µg/mL.

DISCUSSION

Synthesis of CuO NPs was performed successfully using chemical reduction method. The CuO NPs showed remarkable antibacterial activity against *Streptococcus* sp. and *Staphylococcus* sp. A few studies have been performed to reveal the mechanism of bactericidal action of NPs. It is difficult to distinguish between the bactericidal activities of NPs from the ions released by the NPs themselves [16]. Ruparelia *et al*. estimated the concentration of released ions for 10 mg of copper NPs suspended in 100 mL nutrient media and distilled water [17]. They found that the concentration of Cu2+ ions released in nutrient media was 17 mg/L after 24 h of incubation in a rotary shaker, while in distilled water under the same conditions over a period of 24 h, the concentration of ions released was 0.5 mg/L–1 mg/L. These results indicate that the nutrient media can facilitate the release of Cu2+ ions. The considerably greater release of Cu2+ ions in the nutrient media is possibly due to the interaction of the media chloride ions with the oxide layer of the NPs [17]. Consequently, the bactericidal effects observed in this study might have been influenced by the release of Cu2+ ions in solution. The presence of NPs in suspension would ensure continuous release of ions into the nutrient media [18]. There are a few mechanisms of NPs toxicity suggested by other works. For example, copper ions released by the NPs may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death [19]. Copper ions inside the bacterial cells may bind to deoxyribonucleic acid molecules and form cross-linking within and between the nucleic acid strands, resulting in the disorganized helical structure. In addition, copper ion uptake by the bacterial cells has also been found to damage important biochemical processes [20,21].

In the study conducted by Jeyaraman *et al.,* copper NPs displayed antibacterial activity toward the tested pathogenic strains of *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*; in a similar manner, antifungal activity was observed toward *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans,* and respectively. *Bacillus subtilis*

Table 1: Gram staining and colony characteristics of bacterial isolates

Color	Opacity	Consistency	Elevation	Gram nature	Capsule staining
Off white	Opaque	Sticky	Flat	Gram +ve	+ve

Table 2: Biochemical test for the identification of isolates

Isolate	Sugar fermentation	Citrate utilization test	Catalase test	Methyl red test	Blood agar test	Tentative identification
1	Lactose+ve Mannitol+ve	+ve	−ve	+ve	Gamma-hemolysis	*Streptococcus* species
2	Lactose+ve Mannitol−ve	-ve	+ve	+ve	β-hemolysis	*Staphylococcus* species

depicted the highest sensitivity to NPs compared to the other strains and was more adversely affected by the copper NPs that were observed between the inhibition zone observed in disk diffusion test and MIC/ minimum bactericidal concentration determined based on liquid cultures with the various strains [22]. Pawar *et al.* reported the antibacterial activity of CuO NPs against food pathogen *B. cereus* [23]. In another study conducted by Azam *et al.,* it was found that CuO NPs have shown greater antimicrobial activity against *B. subtilis* and *S. aureus*. The variation in the sensitivity or resistance to both Gram-positive and Gram-negative bacteria populations could be due to the differences in the cell structure, physiology, metabolism, or degree of contact of organisms with NPs. For example, greater sensitivity among Gram-positive bacteria such as *B. subtilis* and *S. aureus* to the CuO NPs has been attributed to the greater abundance of amines and carboxyl groups on their cell surface and greater affinity of copper toward these groups [24]. Alternatively, Gram-negative bacteria like *E. coli* have a special cell membrane structure which possesses an important ability to resist antimicrobial agents. Furthermore, other factors such as NP diffusion rates may also affect bacterial strain differently. The study indicates that the CuO NPs inhibit the growth of both Gram-negative and Gram-positive bacteria and the zone of inhibition decreases with the increase in annealing temperature from 400°C to 700°C, the zone of inhibition is maximum when the particle size is minimum (20±1.24 nm). These results demonstrate the excellent antimicrobial behavior of CuO NPs synthesized at low temperature. The interactions between the negative charges of microorganisms and the positive charge of NPs produce an electromagnetic attraction between the microbe and effective levels of active NPs. Such interactions lead to oxidation of surface molecules of microbes resulting in their death.

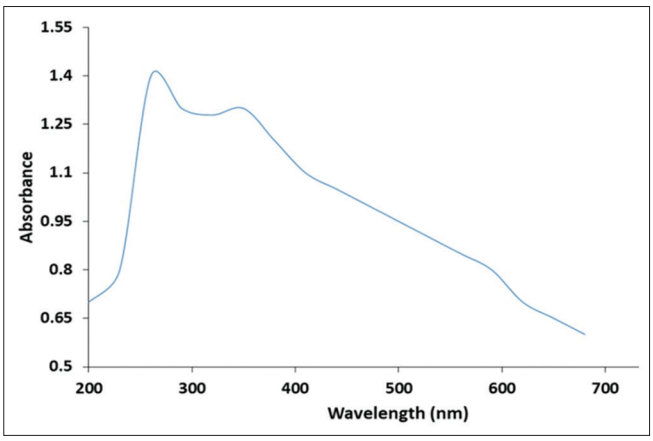

Fig. 1: Ultraviolet-visible spectroscopy of copper oxide nanoparticles

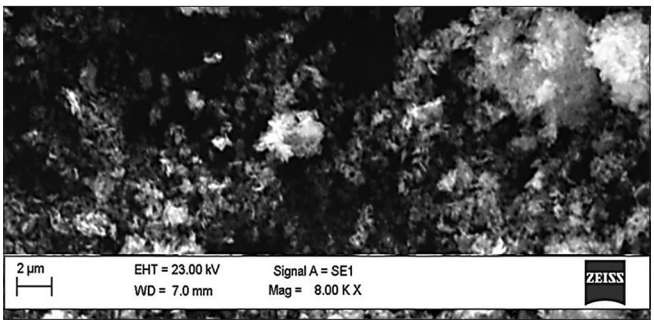

Fig. 2: Scanning electron microscopy images of copper oxide nanoparticles

CONCLUSION

CuO NPs were synthesized by chemical precipitation method. CuO NP is characterized by UV-visible spectroscopy and SEM. Synthesized CuO NP has shown antimicrobial activity against *Staphylococcus* and *Streptococcus* sp. Considering increased antibiotic resistance in pathogenic microorganisms, CuO NP should become cost-effective alternative for the development of antimicrobial agent against pathogenic microorganisms.

AUTHORS' CONTRIBUTIONS

SS, DP, and NJ contributed in experimental data generation, data analysis, and collated the published literature in similar domain. VT, JP, and RH guided the work, drafted the manuscript, and critical revision. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

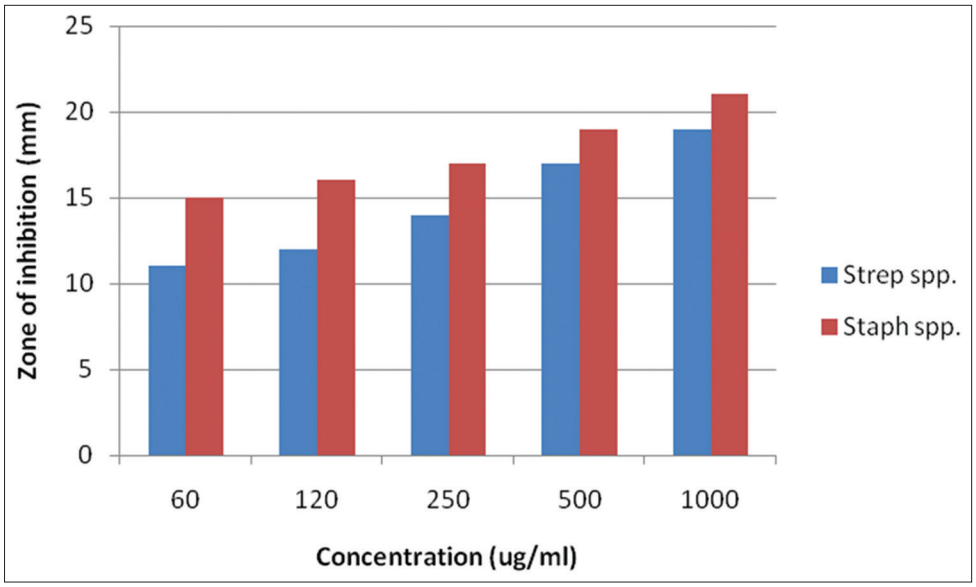

Fig. 3: Comparison of antimicrobial activity of isolates using various copper oxide nanoparticle concentrations

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