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Effect of copper oxide nanoparticles as an Anti-bacterial in inhibiting the growth of *E.coli*

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Abstract
The present study focuses on investigating the inhibitory efficacy of copper oxide nanoparticles against the bacterial strain *Escherichia coli*, with the aim of evaluating their potential as antibacterial agents. Additionally, the research aims to determine the optimal concentration of zinc oxide nanoparticles required to effectively suppress the growth of *E. coli*. The study demonstrated the efficacy of zinc nanoparticles in inhibiting the growth of *E. coli* colonies cultivated on Petri plates. The concentrations and percentages evaluated in this investigation were 10%, 25%, 50%, 75%, and 100%, respectively. The minimal concentration of zinc oxide nanoparticles that is deemed effective as an antibacterial agent in suppressing the growth of *E. coli* bacteria is 50%. At this concentration, it is evident that the bacteria will not proliferate within a specific region on the plate. The inhibitory capacity exhibits an upward trend as the concentration of zinc nanoparticles escalates until it attains a level of 100%, which is deemed the optimal concentration, this concentration is juxtaposed with the antibiotic (cefotaxime), known for its efficacy in suppressing the proliferation of E. coli bacteria. The work demonstrates that the magnitude of the retarding area is 20 millimeters. Hence, based on the findings of this study, copper oxide nanoparticles can be regarded as a viable antimicrobial agent, demonstrating efficacy in combating various infections and diseases caused by E. coli. This efficacy is comparable to that of the antibiotic cefotaxime (CX30) in terms of inhibitory activity. The observed outcome and inhibitory efficacy in suppressing the proliferation of this particular strain of bacteria were identical.

Keywords: Copper oxide nanoparticles**,** *E.* coli**,** anti-bacterial activity, Cefotaxime CX30

Introduction
Nanoparticles have become a subject of extensive research and application across various scientific disciplines, including medical, pharmaceutical, biological, industrial, and military fields. Their significance has attracted the attention of numerous researchers and scientists, leading to their inclusion in a wide range of studies within diverse scientific, cognitive, and applied domains. Materials and nanoparticles can be divided into several categories based on their size, shape, physical qualities, chemical composition, and engineering structures. Various categories of nanotechnologies can be distinguished based on the specific characteristics of the research work being conducted. These categories include metallic nanoparticle derivatives, carbon nanoparticles, ceramic nanoparticles, polymer nanoparticles, and semiconductor nanoparticles. Metallic nanomaterials and semiconductors have gained significant attention in various fields, particularly in biomedical research [1]. These materials are utilized in the production of medications, sensors, and cancer treatment, as well as in the realm of paints [2]. Additionally, they find applications in electronics [3] and exhibit antibacterial properties [4]. Furthermore, their potential applications extend to diverse areas, contingent upon the specific focus of research and experimental investigations, such as sensor development. Among the researchers and scientists who have manufactured them, silver nanoparticles (Ag-NPs) and copper oxide nanoparticles (CuO-NPs) are regarded as the most extensively utilized. Numerous interconnected devices and materials are involved in various applications, including the synthesis of antibiotics and the fabrication of medical, biological, and biochemical detection instruments. A wide range of chemical and organic compounds exhibit antibacterial properties, such as penicillin, which is classified as a member of the beta-lactam group. Additionally, some natural substances have been identified as effective agents in the eradication or suppression of microbial organisms [5]. Research in the field of nanotechnology places significant emphasis on metals and semiconductors, as evidenced by studies employing nanoparticles [6]. It is important to acknowledge that certain chemical compounds, such as organic hydrogen peroxide (OHP), exhibit reactive behavior when associated with nanoparticles. This reactivity manifests in the deposition of nanoparticles on the external cellular membrane of microorganisms, as well as the accumulation of nanoparticles within the cytoplasm of bacterial species. Consequently, this phenomenon results in the demise of minute living organisms [7]. Furthermore, various categories of nanocomposites have been observed to exert detrimental effects on the constituents of living cells, such as lipids, peptidoglycans, proteins, and nucleic acids. These nanocomposites induce alterations in the chemical composition, resulting in diverse biological activities. Moreover, their lack of sequence and interference with biological processes contribute to their disruptive nature [8]. Nanoparticles generated from a range of metals and some semiconductors are widely recognized as fundamental and significant materials due to their extensive utilization in the domains of medicine and nanotechnology. In the realm of scientific research, numerous metal oxide nanoparticles, including zinc oxide, have been identified as crucial components due to their ability to fulfill specific functions. Additionally, copper compounds have garnered significant

attention due to their diverse chemical and physical properties, which enable their utilization in various activities, notably owing to their high electrical conductivity. In addition to elevated temperatures, their impact on augmenting electronic correlation, kinetic, and rotational dynamics has been seen [9]. The introduction of various microorganisms into the air, water, and soil can give rise to a multitude of issues and disparities in living conditions, hence posing significant risks within the realm of healthcare. In light of the widespread emergence of antibiotic-resistant bacteria and their associated pathogenic infections, scientists have undertaken investigations into alternative antimicrobial agents, including metallic nanoparticles and antimicrobial peptides [10]. Numerous studies and research endeavors have demonstrated the potential application of metal nanoparticles as inhibitors to impede the proliferation of bacteria and other microbial entities. Among these nanoparticles, copper oxide has emerged as a noteworthy candidate, exhibiting significant efficacy in effectively curbing the growth of *E. coli* bacteria.

Materials and Methods

The sequence of steps is an essential and important factor in the completion of our current research, based on the provision of a number of materials and thematic devices necessary to be present during the research work. These materials are: CuO-NPs with a diameter of about (50. nm). The culture media used are MacConkey agar, Nutrient agar and Muller-Hinton agar, in addition to the antibiotic tablet cefotaximeCX30. Samples of bacterial isolates were taken from patients of Al Hindiya General Hospital in Karbala, Iraq. These isolated bacterial samples were grown on MacConkey agar plates for a period ranging between (24 and 48) hours at a temperature of 37°C during bacterial isolation and purification. By Viteck's method [2]. CuO NPs were used as an antibacterial that inhibits the growth of Gram-negative bacteria *E. coli*. In order to know the fixative ability of CuO NPs against the gram-negative bacteria *E. coli*, the test is carried out in the clinical laboratory, to determine the extent of bacterial resistance to the antibiotics used for comparison, as well as to CuO-NPs, by knowing the area in the region of inhibition and documenting it. The concentrations used for CuO-NPs are (10.%, 25.%, 50.%, 75.%, 100.%), dilution is done in distilled water. The isolates were incubated for 24 hours at (37°C). The test results were recorded when the zone of inhibition was observed after the incubation period, and then the diameter of the inhibition zone was measured. Approximately (10 ml) of tube broth medium was prepared, and then each sample was sterilely inoculated with (1 ml) of its own bacterial suspension. Five dilutions of copper nanoparticles: (10.%, 25.%, 50.%, 75.%, 100.%) were prepared in D.W. The inoculated groups were incubated in (37 degrees Celsius). After the incubation period, visible turbidity in each tube, was investigated, show table no: (1).

Table 1: The table shows the value of different concentrations, and their activities on inhibition of the bacterial growth).

CuONPs concentrations% with controle	The measure of inhibition zones mm
10%	non
25%	non
50%	5mm
75%	10mm
100%	20mm

Copper oxide particles have a group of chemical and physical properties that serve as an antibacterial, in it is considered one of the metals with strong conductors of high temperatures, strong electronic effect, in addition to spin dynamics. Because of the large spread by antibiotic-resistant strains that lead to infections, we used alternatives to antibiotics as anti-bacterial and anti-microbial agents, and among these alternatives are nanoparticles as their weight serves the desired purpose [10].

Results and Discussion

The antibacterial efficacy of copper oxide nanoparticles refers to their capacity to eradicate and inhibit the proliferation of specific bacterial strains, while concurrently avoiding any detrimental effects on the surrounding infected tissues. Furthermore, these nanoparticles exhibit a lack of cytotoxicity towards the tissues and cells present at the site of infection [11]. The present study employed copper oxide nanoparticles to effectively suppress the proliferation of Escherichia coli germs, as evidenced by the outcomes of clinical efficacy testing. Various quantities of copper oxide nanoparticles were evaluated, and a comparison was conducted with the antibiotic cefotaxime CX30. Our current research study aligns with a number of prior scientific investigations conducted in this subject [12]. The optimal Concentration of copper oxide nanoparticles, specifically at a level of 50%, has been found to exhibit a notable inhibitory effect on bacterial growth when compared to the antibiotic cefotaxime CX30. This assertion is unequivocally illustrated by the depiction presented in figure (1):

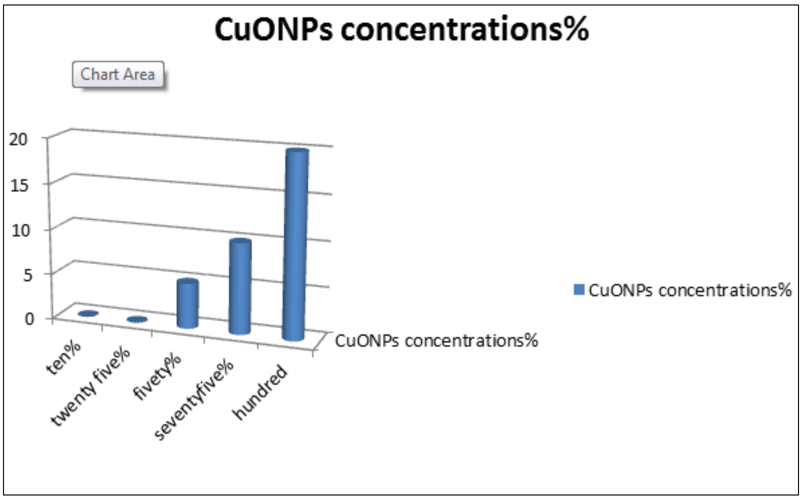

Fig 1: (Illustration of percent. of CuO-NPs affected on the growth of E.coli).

The oxides of most metallic nanoparticles, including magnesium, silver, copper, zinc, iron and nickel, have distinctive properties as anti-microbial agents for various types of microbes. For this reason, our research work includes studying the effectiveness of copper oxide particles as an anti-bacterial, specifically E. coli, as shown in Figure 2, it becomes clear to us Copper oxide nanoparticles have no activity if they are at a concentration of (10-25%), but their Effectiveness starts at a concentration (50%) and above. The areas of inhibition are zero under the influence of both concentrations (10%) and (25%), but when the concentration is increased to reach the concentration (50%), there will be areas of inhibition, until it reaches the full 100% concentration, so that the areas of inhibition reach Approximately (5. mm) on Petri dishes.

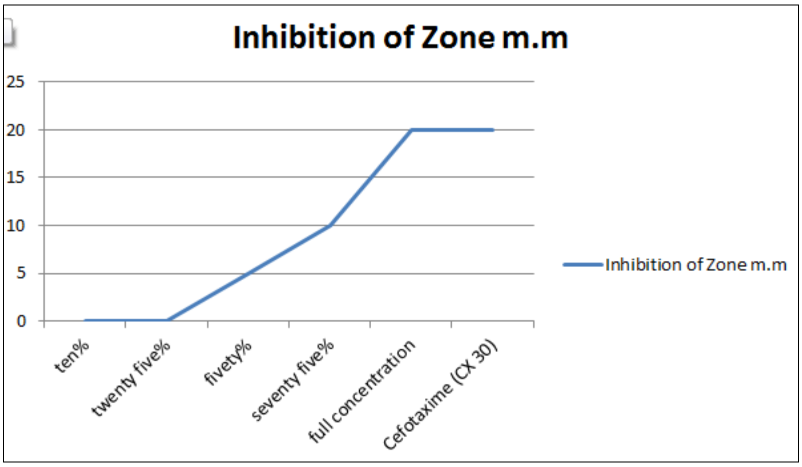

Fig 2: (Illustration of zone of inhibition, against bacterial growth on petri-dish, Which caused by the CuO-NPs activity, by using many concentrations).

Copper oxide nanoparticles are extensively utilized across various disciplines due to their multifunctional properties. Notably, these nanoparticles exhibit a substantial specific surface area, enhanced electrical conductivity, elevated hardness and ductility, in addition to the inherent characteristics of copper oxide nanoparticles. Furthermore, there are additional features associated with it, such as its anti-bacterial and anti-microbial attributes. Copper nanoparticles have found extensive application in various industries, including catalyst production, sensor development, plastic and metal coating manufacturing, solar energy battery fabrication, electrical transformer production, and conductor utilization [13]. The utilization of copper oxide nanoparticles has witnessed a notable rise, prompting the development of novel approaches aimed at safeguarding the environment and mitigating potential hazards to public health and ecological systems. However, it is crucial to acknowledge that the uncontrolled release of these nanoparticles can have detrimental consequences, one of which is the inhibition of denitrification processes in wastewater treatment systems [14]. It is clear to us from what has been said that the appropriate concentration to kill and inhibit the growth of bacteria is 50%, and the higher the concentration of copper oxide nanoparticles, the higher their receptivity and effectiveness as an inhibitor, so that they have the same effect as the aforementioned antibiotic. The area of inhibition formed in preventing the growth of bacteria is estimated at (20 mm). As for the other concentrations used for this purpose, they are less effective in inhibiting the growth of bacteria on the Petri dishes on which the bacteria grew.

The chemical and physical properties possessed by copper nanoparticles are what enabled them to be usable and useful in many applications in all industrial, medical and biological fields such as the manufacture of catalysts, sensors, and electrode materials, as well as the manufacture of field emitters (FE), magnetic storage media, photovoltaic devices, and electrode materials that have been applied in the manufacture of lithium-ion batteries, among others. It is possible to use copper oxide nanostructures and copper oxide nanoparticles in the decomposition of nitrous oxide, the catalytic reductions that are selective for nitric oxide. There are various forms of copper oxide nanostructures used in the production of nanorods [15] nanowires, nanoneedles, (nanobelts - nanoribbons), nanotubes, nano bres using various synthetic techniques. Copper oxide nanostructures, or copper oxide nanoparticles, and various forms of it have also been manufactured for various purposes such as the manufacture of nanosheets, nanofilaments, as well as other industries, such as the manufacture of nanospheres. The use of copper oxide nanoparticles has an economic benefit as an alternative material for antibiotics and bacteria, as well as its use in the treatment of infections that are outside the uterus, without any risk, and the environment for the development of antibiotic-resistant strains of bacteria.

Conclusion

We conclude from what has been mentioned in our current research that copper oxide nanoparticles can be used in the medical, industrial and biological fields, because of their physical and chemical properties that enable them to perform multiple functions compared to other materials, so we can consider them as an appropriate alternative to the antibiotic (cefotaxinCX30) because that Copper nanoparticles performed the required purpose in inhibiting the growth of bacteria E, and the appropriate and effective concentrations of copper oxide nanoparticles are 50% and 100%. On this basis, we can consider that copper oxide nanoparticles are an effective drug against infectious and inflammatory diseases caused by *E. coli*.

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