Research Article

Biosynthesis of Copper Oxide Nanoparticles Using *Streptomyces* MHM38 and Its Biological Applications

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Received 8 November 2020; Revised 7 December 2020; Accepted 18 December 2020; Published 4 January 2021

Academic Editor: Shahid Ali

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Biosynthesis methods employing microorganisms have emerged as an eco-friendly, clean, and viable alternative to chemical and physical processes. The present study reports the synthesis of copper oxide nanoparticles (CuONPs) using cell-free culture supernatant of marine *Streptomyces* sp. MHM38. For the optimized production of CuONPs, the influence of some parameters, such as the concentration of copper sulfate (CuSO_4), reaction time, filtrate to substrate ratio, and pH, was studied. 5 mM of CuSO_4 was optimal for nanoparticle (NP) production. Well-defined CuONP formation occurred after 60 min of incubation when an equal volume of filtrate (cell-free supernatant) to substrate (CuSO_4 solution) was added. UV-visible spectroscopy analysis of CuONPs exhibited a peak at 550 nm, which corresponds to the surface plasmon resonance of CuONPs. Most of the particles were spherical and were 1.72–13.49 nm when measured using a transmission electron microscope. The antimicrobial activity of CuONPs was determined using a well diffusion method against *Enterococcus faecalis* ATCC 29212, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8939, fungi (*Rhizoctonia solani*, *Fusarium solani*, and *Aspergillus niger*), and yeast (*Candida albicans* ATCC 10237). The highest antimicrobial activities were recorded against *Candida albicans* ATCC 10237, whereas *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 8939 showed the less activity. The biochemical findings of the CuONP groups were significant ($p < 0.05$) with diminished levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total and direct bilirubin, urea, and creatinine compared with the paracetamol group. Nonenzymatic and enzymatic antioxidants of the CuONP groups were significantly elevated ($p < 0.05$) in SOD and GSH levels, and exceptionally low nitric oxide (NO) and malondialdehyde (MAD) levels were found for the paracetamol group. The histopathological examination of the CuONP groups assured the impact of improving CuONPs against paracetamol-induced liver damage.

1. Introduction

Metal nanoparticles are synthesized and used because of their unique electrical, optical, catalytic, and magnetic characteristics [1–3], which differ from the characteristics of bulk materials. Recently, many chemical and physical methods have achieved the synthesis of inorganic nanoparticles. However, biological synthesis is realized globally as being dangerous, expensive, and not an environmentally friendly chemical

process [4, 5]. Therefore, it is essential to develop fast, cost-effective, ecological friendly, and easy-to-scale synthetic approaches of manufacturing nanoparticles of metal using biological systems. It is well established that in harsh conditions microbes develop mechanisms to survive in toxic metals by turning toxic metal ions into their corresponding nontoxic forms of metal sulfide/oxide [6]. Many of the destructive impacts of physical and chemical methods can be resolved by the green synthesis of NPs using various

biological entities. These involve the mild pH, pressure, and temperature for biosynthesis of NPs and do not include harmful or hazardous substances and prohibit the addition of external reducing, capping, and stabilizing agents [7]. The specifics of nanotransformation mechanisms are not well known. In the case of copper, a wide range of biological tools, such as bacteria [8, 9], fungi [10], algae [11], and plants [12], can be used to synthesize nanoparticles. A substantial proportion of the world's population uses nanoparticles to treat some diseases. Recent studies have indicated that CuNPs have broad biological properties [13]. Actinomycetes are a diverse group of Gram-positive bacteria that are common in soil and widely distributed in different environments, and they are known to generate many forms of novel secondary metabolites [14]. The bioactive compounds of actinomycetes are active against bacteria, fungi, and viruses [15]. Actinomycetes are highly regarded as being important candidates for metal nanoparticle synthesis [16]. They are stable and polydisperse, making them an effective candidate for intracellular and extracellular metal nanoparticle synthesis. The electron binding of Ag^+ ions to a mycelial cell wall enzyme carboxylate group results in intracellular nanoparticle synthesis [17–19]. *Streptomyces* are a commonly used actinomycetes species in nanoparticulate biosynthesis [20], and they have been documented to produce silver, gold, manganese, copper, and zinc nanoparticles [21, 22]. Copper nanoparticles (CuNPs) have important applications in various fields [23–25]. Copper oxide nanoparticles (CuONPs) have essential antimicrobial properties by inhibiting bacterial, fungal, viral, and algal growth [26, 27]. Nanosized copper oxide has a longer shelf life than other organic antimicrobials, such as silver and gold [28]. Copper oxide nanoparticles synthesized with actinomycetes show antibacterial properties [29]. Paracetamol is a medication with antipyretic and analgesic impacts that is broadly utilized by the wider community and taken freely without supervision. However, high doses of paracetamol can cause liver damage. Paracetamol is expected to be a significant factor causing severe liver damage [30]. This study was designed to use a marine actinobacterial *Streptomyces* sp. strain that was screened for biosynthesis, optimization, and characterization of produced copper oxide nanoparticles (CuONPs), which were evaluated for their acceptability to animal tissue, antioxidant and hepatoprotective activity, and inhibitory action against some pathogenic microorganisms.

2. Material and Method

2.1. Microorganisms and Cultural Conditions. *Streptomyces* sp. MHM38 was isolated from a marine sediment sample in the Suez gulf and deposited in GenBank as *Streptomyces* sp. MHM38 with accession number KU764745 by Dr. Moaz M. Hamed. This marine actinobacterial isolate was maintained on slant containing starch nitrate agar medium (SNM) with a specific composition (g/l: starch, 20; K_2HPO_4 , 1.0; KNO_3 , 2.0; MgSO_4 , 0.5; and agar 18.0; H_2O , 1.0l). Components were dissolved in 0.5l of distilled water and 0.5l of seawater [31]. Measures of 50 and 20 $\mu\text{g ml}^{-1}$ tetracycline and nystatin, respectively, were applied as antibac-

terial and antifungal agents to prevent bacterial and fungal infection following autoclaving and solidification. The strain was incubated for seven days at 30°C–32°C. The isolate was stored as spore suspension in 20% (v/v) glycerol at –20°C for subsequent investigation.

2.2. Inoculum Preparation. A 250 ml Erlenmeyer flask was used containing 50 ml of SNM. This flask was inoculated with old stock culture and incubated for five days in a rotator incubator shaker at 30°C–32°C and 200 rpm, and it was used as an inoculum for subsequent experiments.

2.3. Extracellular Synthesis of CuONPs. The isolate was freshly inoculated in an Erlenmeyer flask containing 50 ml of the abovementioned production medium to screen *Streptomyces* sp. MHM38 for the synthesis of CuONPs. The culture was centrifuged at 10,000 rpm at the end of the incubation, and supernatants were used to detect copper nanoparticles. The supernatants (biomass filtrate) were used for green synthesis of CuONPs. A volume of 15 ml of 1 mM CuSO_4 was added to 15 ml of the isolate supernatant in 100 ml Erlenmeyer flasks. The flasks were incubated at 30°C–32°C and observed for color change. Two controls were used: the first control (sterile media mixed with 1 mM copper sulfate) was used to establish that media components cannot reduce copper ions to copper nanoparticles. In the negative control (copper sulfate solution), no color change was observed over time. The flasks were monitored daily for any visual color change. UV-visible spectroscopy in the range of 200–800 nm was conducted for flasks with color adjustment [4].

2.4. Optimization of Different Factors on the Production of Copper Oxide Nanoparticles by *Streptomyces* sp. MHM38

2.4.1. Effect of Copper Concentration on Nanoparticle Production. 15 ml of cell-free supernatant was added to 15 ml of 1 to 10 mM CuSO_4 . The blend was incubated as above, and UV-vis spectroscopy was used to study CuONPs.

2.4.2. Effect of Reaction Time on Nanoparticle Production. Nanoparticle synthesis and stability are influenced by reaction time. 15 ml of cell-free supernatant was added to 15 ml of 5 mM CuSO_4 solution. The mixture was incubated for various periods as above, and a UV-visible spectroscopy analysis was performed to analyze CuONPs.

2.4.3. Effect of the Substrate to Filtrate Ratio on Nanoparticle Production. Three flasks were prepared to study the effect of different substrate to filtrate ratios on CuONP formation: in the first, 15 ml of cell-free supernatant was added to 15 ml of 5 mM CuSO_4 solution; in the second, 15 ml of cell-free supernatant was added to 7.5 ml of 5 mM CuSO_4 solution; and in the third, 15 ml of cell-free supernatant was added to 30 ml of 5 mM CuSO_4 solution. The mixture was incubated statically for 60 min, and the formed CuONPs were analyzed using UV-visible spectroscopy.

2.4.4. Effect of pH on Nanoparticle Production. Cell-free supernatant was exposed to 5 mM CuSO_4 at pH of 6, 7, and 8 and incubated for 60 min to investigate the impact of pH

on CuONP production. UV-visible spectroscopy was used to analyze the formed CuONPs.

2.5. Characterization of Copper Oxide Nanoparticles

2.5.1. UV-Visible Spectral Analysis. Color changes were observed for biosynthesized copper oxide nanoparticles using the cell-free supernatant. CuONPs were characterized using UV-visible spectroscopy (Double Beam Spectrophotometer 6800, JENWAY) in the range of 200–800 nm, at regular intervals.

2.5.2. Transmission Electron Microscope Analysis. CuONP solution was diluted and sonicated. A drop was placed on a carbon-coated grid, and water was evaporated. Measurements were performed on a JEM-100-CX at a voltage acceleration of 80 KV dry. Then, samples were examined with a transmission electron microscope (TEM) at the Faculty of Science, Alexandria University.

2.5.3. Energy Dispersive X-Ray Spectroscopy Analysis. The technique described by Jyoti et al. [32] was used to determine the elementary structure of samples and confirm that the nanoparticle suspension contained only copper [33]. This analysis was conducted using the powder of lyophilized CuONPs. A sample was analyzed using the Oxford instrument attached to a scanning electron microscope at the Electron Microscope Unit, Faculty of Science, Alexandria University.

2.5.4. X-Ray Diffraction Analysis. CuONP samples were dried for X-ray diffraction (XRD) pattern analysis, which was recorded in the transmission mode on a Shimadzu XRD7000 instrument (at the Central Laboratory, the City of Scientific Research and Technological Applications, Egypt) operating at 40 KV current 30 mA with Cu K α radiation ($\lambda = 1.5404 \text{ \AA}$) [34]. A monochromatic X-ray beam with a lambda wavelength was used to analyze the crystalline nature of the samples [35].

2.6. Biotechnological Application of Copper Nanoparticles

2.6.1. Antimicrobial Activity of Copper Nanoparticles Using the Agar Diffusion Method. Biosynthesized CuONPs were examined for antimicrobial activity against Gram-positive bacterial pathogens (*Enterococcus faecalis* ATCC 29212), Gram-negative bacteria (*Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 8939), fungi (*Rhizoctonia solani*, *Fusarium solani*, and *Aspergillus niger*), and yeast (*Candida albicans* ATCC 10237) using the well diffusion method: Mueller-Hinton Agar was used for bacteria, Sabouraud Dextrose Agar was used for *C. albicans*, and potato D-glucose agar was used for fungi. A 100 μl bacterial suspension was used to prepare bacterial lawns for each bacterial test organism. These bacterial pathogens were kindly provided by the staff members of the National Institute of Oceanography and Fisheries (NIOF), Alexandria branch, and the Assiut University Mycological Center (AUMMC), Assiut, Egypt, also supplied fungal cultures. An 8 mm diameter agar well was made using a sterilized cork borer in stainless steel. The wells were loaded with

100 μl of 200 mg/ml concentrations of CuONP solution and 100 μl of culture broth from *Streptomyces* sp. MHM38 cell-free supernatant, and DMSO was used as a solvent. The plates were incubated for 24 h at 37°C for bacteria and 120 h at 28°C for fungi. Then, they were examined for inhibition zones. The diameter of inhibition areas was measured, and the mean value was recorded in millimeters for each organism [36].

2.6.2. Effect of Copper Oxide Nanoparticles against Oxidative Stress

(1) Animal and Experimental Design. Eighty healthy, eight-week-old, male albino Sprague-Dawley rats weighing 180–200 g were obtained and housed in the biology lab in the Agricultural Chemistry Department, Faculty of Agriculture, Minia University, at a controlled temperature of $25^\circ\text{C} \pm 2^\circ\text{C}$ with a 12 h dark/light photoperiod for an adaptation period of two weeks. The study protocol was approved by the Agricultural Chemistry Department Ethics Committee, Minia University Faculty of Agriculture. Rats were randomly divided into eight groups with 10 rats in each group and subjected for 21 days to one of eight treatments: control (group 1), administered orally with 500 mg/kg b.wt paracetamol (PAC) daily for the last five days [37] (group 2), administered orally with 1 mg of CuONPs/kg b.wt daily for 21 days (group 3), administered orally with 2 mg of CuONPs/kg b.wt daily for 21 days (group 4), administered orally with 5 mg of CuONPs/kg b.wt daily for 21 days (group 5), administered with 1 mg of CuONPs/kg b.wt for 16 days and then received both CuONPs and paracetamol in the last five days (group 6), administered with 2 mg of CuONPs/kg b.wt for 16 days and then received both CuONPs and paracetamol in the last five days (group 7), and administered with 5 mg of CuONPs/kg b.wt for 16 days and then received both CuONPs and paracetamol in the last five days (group 8). After 24 hours, rats were sacrificed under light ether narcosis followed by decapitation to obtain biomaterials (blood and liver) for research. The blood sample was collected from the portal vein in the tubes that do not contain anticoagulants. The blood samples obtained were centrifuged at 1200 g for 15 min to separate the serum. The serum obtained was used to conduct biochemical analyses of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total and direct bilirubin, urea, and creatinine, which were measured according to El-Naggar and Abdelwahed [36], Al-Rubaei et al. [37], Davies et al. [38], Montgomery and Dymock [39], Ohkawa et al. [40], and Kakkar et al. [41], respectively. The liver was homogenized in homogenization buffer PBS (pH 7.4), and then, homogenates were centrifuged at 10,000 g for 30 min ($+4^\circ\text{C}$) to obtain supernatants. The supernatants of liver tissues of rats were used to analyze glutathione (GSH) levels following Davies et al. [38], nitric oxide (NO) following Montgomery and Dymock [39], malondialdehyde (MDA) following Ohkawa et al. [40], and superoxide dismutase (SOD) following Kakkar et al. [41]. Liver tissues were also fixed in neutral buffered 10% formalin, dehydrated, cleared, and paraffin ionized for paraffin blocks, and 5-micron sections were obtained,

mounted on a glass slide, and stained with hematoxylin and eosin and Prussian blue reaction [42].

(2) *Statistical Analysis.* Data are presented as the mean $\pm$ standard error of the means (SEM). One-way analysis of variance followed by Tukey post hoc test using the Statistical Package for the Social Sciences (SPSS software V. 18.0) was performed to compare groups. p values < 0.05 were considered significant.

3. Results and Discussion

3.1. Evaluation of Biosynthesis of Copper Oxide Nanoparticles Using *Streptomyces* sp. MHM38. *Streptomyces* sp. MHM38 reduced the copper ions to copper nanoparticles. The biosynthesis of CuONPs was indicated by changing the soft blue reaction blend to green after 1% (v/v) of 1 mM aqueous CuSO_4 was added to the cell-free *Streptomyces* sp. MHM38 supernatant. By comparison, no color change was observed in aqueous CuSO_4 incubated under the same conditions without cell-free supernatant (Figure 1). The formation of colors depends on the surface vibration of plasmon [4, 43]. Our results agree with Shantkriti and Rani [4], who mentioned that when cell-free supernatant of *Pseudomonas fluorescens* was added to CuSO_4 solution and incubated for 48 h the color of the reaction mixtures changed from blue to dark green. Therefore, isolate CA-1 was considered the most potent isolate by endophytic actinomycetes due to color changes and maximum absorption peaks [44].

3.2. UV-Visible Spectral Analysis. The presence of nanoparticles was confirmed by UV-visible spectrophotometry within the 200–800 nm range the CuONPs formed by the *Streptomyces* sp. MHM38 which showed an absorption peak of 550 nm (Figure 2) of the different surface plasmon resonance (SPR) spectra, indicating the existence of CuONPs. Depending on individual particle properties, such as size, shape, and capping agents, the exact location of the SPR band can vary [45]. Brause and his team [46] reported that SPR is dominant in the optical absorption of metal nanoparticles, and particle size is linked to the absorption pick. The SPR of CuONPs in aqueous solution increases to longer wavelengths, with particle size increasing. The position and form of copper nanocluster absorption of plasmon are strongly dependent on particle size, stabilizing molecules or surface adsorbed particles, and the media's bioelectricity [47]. Our results agree with Ghorbani et al. [48], who mentioned that the copper SPR band of *Salmonella typhimurium* occurred at 565 nm [4]. The copper SPR band of *Pseudomonas fluorescens* exhibits a distinct absorption peak in the region of 550–650 nm.

3.3. Optimization of Copper Oxide Nanoparticles Using Box-Behnken Design

3.3.1. Effect of Copper Concentration on Nanoparticle Production. From Figure 3, it is clear that the rate of formation of CuONPs increased with increasing substrate concentration, reaching its maximum at 5 mM of CuSO_4 . The addition of various concentrations of CuSO_4 solution to the pellet did not reveal any color change. Our results agree with

FIGURE 1: Visible observation of copper oxide nanoparticle biosynthesis by *Streptomyces* sp. MHM38 (left) 1 mM CuSO_4 solution (right).

Shantkriti and Rani, who mentioned that the color of the reaction mixtures changed from blue to dark green after 5 mM CuSO_4 was added to the supernatant of *Pseudomonas fluorescens* [4], and they were similar to a finding of a dark green solution when 5 mM of CuSO_4 was added to a flask containing *Morganella* sp. [49].

3.3.2. Effect of Reaction Time on Copper Oxide Nanoparticle Production. Reaction time is essential to the synthesis and stability of nanoparticles. Absorption at 550 nm was shown to increase progressively to 60 min, so there was no change (Figure 4). This suggests that CuONP development increased and size decreased over time. At the same time, Kimber et al. mentioned a complete reduction of CuSO_4 solution to CuNPs by *Shewanella oneidensis* after 96 h [50], and Hamid said that the CuNPs of *Salmonella typhimurium* formed after 20 min [48]. However, Shantkriti and his team [4] found that CuONPs formed using *Pseudomonas fluorescens* 90 min after adding 5 mM of CuSO_4 solution.

3.3.3. Effect of a Substrate to Filtrate Ratio on Copper Oxide Nanoparticle Production. Different volumes of CuSO_4 solutions have been used to understand the CuSO_4 volume required for the efficient production of NPs. When samples were taken at 60 min, the ratio of filtrate (cell-free supernatant) to substrate (CuSO_4 solution) was 1 : 1; the absorbance of CuONPs at 550 nm gave a high value compared with the ratio of filtrate (cell-free supernatant) to substrate (CuSO_4 solution), which was 1 : 1/2, or ratio of filtrate (cell-free supernatant) to substrate (CuSO_4 solution), which was 1 : 2, as shown in Figure 5.

3.3.4. Effect of pH on Copper Nanoparticle Production. Altering pH is thought to help control the shape and size of nanoparticles [51]. The peaks of an acidic pH of 6 were not typical of CuONPs (Figure 6). The alkaline pH provided a high absorption peak at 550 nm, and a characteristic peak of CuONPs was formed by *Pseudomonas fluorescens* at a neutral pH. After optimization, we estimated that the optimum conditions for CuONPs were a substrate concentration of 5 mM. An equal volume of filtrate and substrate was added,

FIGURE 2: UV-visible absorption spectrum of copper nanoparticles synthesized using 15 ml of cell-free *Streptomyces* sp. MHM38 supernatant of 72 h old culture added to 15 ml of 1 mM CuSO_4 solution and incubated statically.

FIGURE 3: Effect of different copper sulfate (CuSO_4) concentrations on the production of copper oxide nanoparticles by *Streptomyces* sp. MHM38.

the pH was adjusted to eight, and the mixture was incubated statically for 60 min [4]. As showed in Figure 7, the color of the solution changed to green when we applied the preview conditions, indicating high levels of production of CuONPs.

3.4. Characterization of Copper Oxide Nanoparticles

3.4.1. TEM. Many studies have classified the shape and size of copper nanoparticles using TEM structures [52]. The present work revealed the spherical form of nanoparticles in the TEM images of copper nanoparticles (Figure 8). TEM analysis of CuONPs produced using *Streptomyces* sp. MHM38 showed that they were relatively uniform in shape. In general, spherical particles appeared with an average dimensional size of 1.72–13.49 nm, which is smaller than the CuNPs formed by *Pseudomonas fluorescens*, showing an extent of 20–80 nm [4]. Kimber et al. [50] mentioned that *Shewanella oneidensis* produced spherical CuNPs in the range of 20–50 nm.

3.4.2. XRD Analysis. The XRD pattern of nanoparticles showed intensive peaks throughout the two-party scope of the value 20–80, similar to the Bragg's copper oxide nanoparticle reflection. Thus, the reaction mixture indicated the formation of copper oxide nanoparticles. CuONPs produced using *Streptomyces* sp. MHM38 distinguished XRD peaks with 2θ values of prominent XRD peaks with 2θ values of 35°, 38°, 48°, 53°, 58°, 65°, 67°, and 72° which were observed (Figure 9). These peaks are assigned to the (111), (202), (020), (202), and (113) reflection planes of face-centered-cubic (fcc) copper, respectively. Our results agree with Chen et al. [53], who mentioned that the XRD pattern of the copper nanoparticles synthesized using N,N'-di-carboxy methyl perylene diimide (PDI) functionalized CuO nanocomposites showed at 2θ values of 35°, 38°, 48°, 53°, 58°, 65°, 67°, and 72°, corresponding to XRD planes (111), (202), (020), (202), and (113) Bragg's reflection based on the fcc structure of CuONPs.

FIGURE 4: Effect of reaction time on the production of copper oxide nanoparticles using *Streptomyces* sp. MHM38.

FIGURE 5: Effect of the ratio between filtrate (cell-free supernatant) to substrate (CuSO_4 solution) concentration on the production of copper oxide nanoparticles using *Streptomyces* sp. MHM38.

3.4.3. Energy Dispersive X-Ray (EDX) Spectroscopy Analysis. EDX and elementary mapping determined the purity and elemental composition of the nanoparticles. In the current research, EDX spectroscopy analysis was performed for CuONPs produced using *Streptomyces* sp. MHM38 (Figure 10), which confirmed the presence of elemental copper based on the signals. In the EDX spectrum, the nanoparticles displayed a peak at eight keV, which is due to the absorption of copper oxide nanocrystallites corresponding to SPR [54]. The optical absorption band peak for nanoparticles produced by *Streptomyces* sp. MHM38 was in the range of 1 to 9 keV, which is typical for the absorption of copper

oxide nanocrystallites. The primary component observed was copper oxide (90%), and other elements were regarded, such as calcium, phosphate, and carbon. The use of a TEM network triggered the carbon distribution. However, there were other EDX peaks for calcium, phosphorus, and copper (5%, 10%, and 2%, respectively), which represent an essential ingredient in bacterial structural proteins that have functional groups, suggesting that they were mixed precipitates from the centrifuged supernatant/metal solution.

3.5. Antimicrobial Activity. Nanoparticles have an elevated surface-volume ratio, tiny size, and elevated dispersion

FIGURE 6: Effect of pH on the production of copper oxide nanoparticles using *Streptomyces* sp. MHM38.

FIGURE 7: Visible observation of optimized copper oxide nanoparticle biosynthesis by *Streptomyces* sp. (Left: 15 ml of supernatant was added to 15 ml of 5 mM CuSO_4 at pH 8. Right: 15 ml of media was added to 15 ml of 5 mM CuSO_4 at pH 8.)

characteristics that enable them to interact with microbial surfaces. The large surface area of CuONPs enhances their interaction with microbes to perform wide antimicrobial operations [55]. However, the few reports on CuONP antimicrobial research have shown that CuONPs are effective against multiple pathogenic microorganisms [56]. The antimicrobial activity of CuONPs was determined on pathogenic bacterial strains Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212), Gram-negative bacteria (*Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 8939), fungi (*Rhizoctonia solani*, *Fusarium solani*, and *Aspergillus niger*), and yeast (*Candida albicans* ATCC 10237) using the method of well diffusion, and the inhibition area values are shown in Table 1 and Figure 11. In each plate, DMSO as control, cell-free supernatant broth with no CuSO_4 addition is maintained. The highest antimicrobial activity was observed against *Candida albicans* ATCC 10237 and *Pseudomonas aeruginosa* ATCC 9027, whereas a lower activity was found against *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC

8939. These findings are consistent with previous studies that examined *Candida albicans* and *Pseudomonas aeruginosa* antimicrobial activity of CuNPs [56]. The appearance of the inhibition area showed that in these locations there was no growth of bacteria. This shows how biosynthesized CuONPs interact with a smaller part/high surface area, of which CuONPs have been adsorbed onto the surface of the micro-organism cell wall. As a result of this, cell walls that destroy human pathogens were demolished and disrupted by the resistance property of biosynthesized CuONPs. The antibacterial activity of copper metal is licensed by the United States Environmental Protection Agency as an antimicrobial agent [57]. The inhibitory action of CuONPs can be due to their small size and high volume-to-volume surface area, allowing it to interact with the microbial cell membrane [58]. Also, their inhibitory action is related to the production of hydroxyl radicals that ruin the helical structure of DNA by binding it and harm vital proteins by binding amino sulfhydryl and carboxyl amino acid groups and then inactivate necessary enzymes [59]. Santo et al. [60] showed the inhibitory action of CuONPs associated with an inactivated surface protein responsible for transporting material across cytoplasmic membranes and destroying selective permeability. Marine actinomycetes have recently revealed biosynthesis of CuONPs and their applications against pathogenic microbes [61]. Green-synthesized AgNPs by *Penicillium chrysogenum* strain F9 can be used to overcome the resistance pattern of *Candida* spp. and recommended as an anti-*Candida* agent [62].

3.6. The Effect of Copper Oxide Nanoparticles Produced Using *Streptomyces* sp. MHM38 against Paracetamol-Induced Liver and Kidney Damage. Tests for ALT, AST, ALP, and bilirubin are essential in diagnosing the condition of the liver. When liver cells (hepatitis) deteriorate, they are released into the bloodstream and level above the normal range. Urea and creatinine are both metabolic wastes excreted by the kidneys through urine, and only a small amount remains in the blood. If there is a disorder of kidney function, then there is an expansion in these two parameters. LDH is a conspicuous

FIGURE 8: Transmission electron microscope image of produced spherical copper oxide nanoparticles using cell-free supernatant of *Streptomyces* sp. MHM38 (a). Histogram of CuONP size distribution (b).

FIGURE 9: X-ray diffraction pattern with regard to the copper oxide nanoparticles biosynthesized using *Streptomyces* sp. MHM38.

marker and a diagnostic tool for tissue injury. As shown in Table 2, there were no significant differences in ALT, AST, ALP, LDH, total and direct bilirubin, urea, and creatinine between the groups treated with CuONPs compared with the control group. However, these parameters increased with an increasing dose of CuONPs. Conversely, significant increases were demonstrated in the ALT, AST, ALP, LDH, total and direct bilirubin, urea, and creatinine levels in paracetamol-treated rats compared with the controlled value. Meanwhile, pretreatment with CuONPs recorded suppression with these values, as shown in Table 2. These results were in agreement with Ravindran et al. [63], who explained that paracetamol increased levels of ALT, AST, ALP, bilirubin, urea, and creatinine. Paracetamol turned into water-soluble products through drug metabolism enzymes and was excreted in the urine in a therapeutic dose [64]. Excess paracetamol is oxidized by the hepatic cytochrome p450 system (CYP450) to N-acetyl-p-benzoquinone imine (NAPQI),

which is toxic [65]. Detoxification is usually expelled from NAPQI by GSH. GSH depletion occurs when using high doses of paracetamol, and consequently, the toxic NAPQI accumulation binds to cellular proteins via cysteinyl sulfhydryl groups and forms NAPQI-protein adducts [66]. This case results in the formation of reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and a hydroxyl root (OH^-) that impacts the cell membrane and stimulates lipid peroxide and also leads to liver necrosis [67]. Liver cell injuries lead to cellular enzymes leaking into the bloodstream and can be measured in serum. The increase in the production of ROS causes damage to the kidney tissue, resulting in a higher level of urea and creatinine [68]. Farghaly and Hussein [69] reported that paracetamol caused an increase in the level of LDH. Paracetamol works to accumulate Ca^{2+} intracellularly, which activates anaerobic glycolysis and phosphofructokinase, forming lactate and increasing LDH [70]. Returning these enzymes to normal levels demonstrates the protective effect of CuONPs against liver and kidney damage and their ability to regenerate liver and kidney cells, which is in agreement with Zhang et al. [71]. Green-synthesized nanoparticles exhibit a beneficial effect against liver and kidney degradation because the bacteria used in nanoparticle synthesis have medicinal properties. These results are consistent with Ghaffar et al. [72]. The protective activity of CuONPs may be attributed to their role in preventing cellular leakage and losing the functional integrity of the cellular membrane in hepatocytes and kidney.

3.7. The Effect of Copper Oxide Nanoparticles Produced Using *Streptomyces* sp. MHM38 against Paracetamol-Induced Oxidative Stress. The oral administration of biosynthesized CuONPs alone did not induce any evident changes in most biochemical parameters compared with the control group. Paracetamol administration produced a significant increase

FIGURE 10: Energy dispersive X-ray spectrum of copper oxide nanoparticles formed using *Streptomyces* sp. MHM38, showing a peak between 1, 8, and 9 keV.

TABLE 1: Antimicrobial activity of copper nanoparticles produced using *Streptomyces* sp. MHM38.

Microorganism	Inhibition zone diameter (mm)		
	Free supernatant	CuONPs	DEMSO
<i>Enterococcus faecalis</i> ATCC 29212	0.0	20.0 ± 0.3	0.0
<i>Salmonella typhimurium</i> ATCC 14028	0.0	18.0 ± 0.4	0.0
<i>Pseudomonas aeruginosa</i> ATCC 9027	16.0 ± 0.6	20.0 ± 0.5	0.0
<i>Escherichia coli</i> ATCC 8939	0.0	18.0 ± 0.5	0.0
<i>Rhizoctonia solani</i>	0.0	0.0	0.0
<i>Fusarium solani</i>	0.0	0.0	0.0
<i>Aspergillus niger</i>	0.0	0.0	0.0
<i>Candida albicans</i> ATCC 10237	18.0 ± 0.5	22.0 ± 0.5	0.0

FIGURE 11: Antibacterial activity of copper nanoparticles against *Candida albicans* ATCC 10237, *Escherichia coli* ATCC 8939, and *Pseudomonas aeruginosa* ATCC 9027 ((a) cell-free supernatant, (b) copper nanoparticles produced using *Streptomyces* sp. MHM38, and (c) DMSO).

TABLE 2: Effect of copper oxide nanoparticles produced using *Streptomyces* sp. MHM38 on biochemical parameters.

Groups Parameters	Control	PAC	CuONPs (1 mg/kg b.wt)	CuONPs (2 mg/kg b.wt)	CuONPs (5 mg/kg b.wt)	CuONPs (1 mg/kg b.wt)+PAC	CuONPs (2 mg/kg b.wt)+PAC	CuONPs (5 mg/kg b.wt)+PAC
ALT (U/l)	22.53 ± 0.59	82.90 ± 3.06	25.60 ^b ± 0.78	24.40 ^b ± 1.21	26.35 ^b ± 0.64	42.23 ^{ab} ± 1.88	39.50 ^{ab} ± 0.89	52.37 ^{ab} ± 2.42
AST (U/l)	80.80 ± 1.73	223.65 ^a ± 7.59	82.48 ^b ± 6.15	73.65 ^b ± 1.99	85.17 ^b ± 1.85	136.25 ^{ab} ± 2.28	109.65 ^{ab} ± 0.43	153.95 ^{ab} ± 0.84
ALP (U/l)	223.2 ± 3.67	611.1 ^a ± 5.77	229.4 ^b ± 1.09	226.95 ^b ± 3.95	231.80 ^b ± 7.63	338.67 ^{ab} ± 5.27	360.03 ^{ab} ± 25.12	411.87 ^{ab} ± 9.61
Total bilirubin (mg/dl)	0.65 ± 0.03	2.27 ^a ± 0.09	0.75 ^b ± 0.03	0.7 ^b ± 0.06	0.8 ^b ± 0.06	1.23 ^{ab} ± 0.13	0.95 ^{ab} ± 0.03	1.7 ^{ab} ± 0.06
Direct bilirubin (mg/dl)	0.4 ± 0.06	0.99 ^a ± 0.06	0.4 ^b ± 0.12	0.38 ^b ± 0.18	0.45 ^b ± 0.02	0.58 ^{ab} ± 0.02	0.51 ^{ab} ± 0.2	0.7 ^{ab} ± 0.05
Urea (mg/dl)	23.45 ± 1.59	50.97 ^a ± 1.42	24.5 ^b ± 1.10	23.83 ^b ± 0.80	27.13 ^b ± 0.57	33.2 ^{ab} ± 0.35	31.70 ^{ab} ± 0.23	40.15 ^{ab} ± 0.02
Creatinine (mg/dl)	1.25 ± 0.03	2.82 ^a ± 0.03	1.31 ^b ± 0.03	1.30 ^b ± 0.06	1.35 ^b ± 0.03	1.70 ^{ab} ± 0.06	1.58 ^{ab} ± 0.03	1.9 ^{ab} ± 0.11
LDH (U/l)	145.7 ± 0.14	374.4 ^a ± 7.04	155.7 ^b ± 2.59	146.0 ^b ± 1.73	158.25 ^b ± 0.14	262.85 ^{ab} ± 10.59	235.7 ^{ab} ± 2.59	283.5 ^{ab} ± 2.02

Results are expressed as the mean ± standard error ($n = 10$) where the mean is significant at $p < 0.05$. ^aCompared with the control group; ^bcompared with the paracetamol group.

TABLE 3: Effect of copper oxide nanoparticles produced using *Streptomyces* sp. MHM38 on nitric oxide, malondialdehyde, superoxide dismutase, and glutathione.

Groups Parameters	Control	PAC	CuONPs (1 mg/kg b.wt)	CuONPs (2 mg/kg b.wt)	CuONPs (5 mg/kg b.wt)	CuONPs (1 mg/kg b.wt)+PAC	CuONPs (2 mg/kg b.wt)+PAC	CuONPs (5 mg/kg b.wt)+PAC
NO ($\mu\text{mol/g}$ tissue)	46.92 $\pm$ 0.15	66.65 ^a $\pm$ 1.69	51.67 ^b $\pm$ 0.07	51.27 ^b $\pm$ 0.14	51.17 ^b $\pm$ 1.48	60.35 ^{ab} $\pm$ 1.68	54.58 ^{ab} $\pm$ 1.57	61.49 ^{ab} $\pm$ 1.49
MDA (nmol/g tissue)	8.15 $\pm$ 0.53	24.40 ^a $\pm$ 0.23	9.40 ^b $\pm$ 0.46	8.60 ^b $\pm$ 0.40	9.50 ^b $\pm$ 0.25	12.27 ^{ab} $\pm$ 0.28	15.10 ^{ab} $\pm$ 0.06	17.19 ^{ab} $\pm$ 1.88
SOD (U/g protein)	23.58 $\pm$ 2.00	9.84 ^a $\pm$ 1.54	21.34 ^b $\pm$ 0.64	23.61 ^b $\pm$ 0.59	23.05 ^b $\pm$ 1.69	12.65 ^{ab} $\pm$ 0.37	19.41 ^{ab} $\pm$ 1.49	17.13 ^{ab} $\pm$ 1.38
GSH (nmol/g tissue)	4.45 $\pm$ 0.17	1.54 ^a $\pm$ 0.02	4.46 ^b $\pm$ 0.03	4.02 ^b $\pm$ 0.01	4.18 ^b $\pm$ 0.33	3.50 ^{ab} $\pm$ 0.23	3.00 ^{ab} $\pm$ 0.1	2.92 ^{ab} $\pm$ 0.01

Results are expressed as mean $\pm$ SE ($n = 10$) where mean is significant at $p < 0.05$. ^aCompared with the control group; ^bcompared with the paracetamol group.

FIGURE 12: Section of rat's livers.

in NO and MAD content accompanied by a marked inhibition of GSH and SOD activities compared with the control group. The administration of biosynthesized CuONPs led to a significant decrease in NO and MAD content and increased GSH and SOD levels compared with the paracetamol group (Table 3). Nonenzymatic antioxidants (GSH) and enzymatic antioxidants (SOD) in natural conditions regulate free radical removal and production and thus maintain the ROS level. Therefore, this antioxidant protects the body from oxidative stress. Ravindran et al. [63] explain that consuming high doses of paracetamol reduces the activity of these enzymatic antioxidants and makes cells more vulnerable to injury caused by free radicals. Madkour and Abdel-Daim [73]

showed that high doses of paracetamol cause oxidative stress and damage the liver, causing increased levels of MDA and NO and a decrease in the activities of SOD compared with the control group. Cytotoxicity occurs due to oxidative stress when the level of free radicals is increased to the point that cells cannot remove them and prevent their formation. An increased level of MDA and NO and decreased SOD and GSH levels indicate tissue damage and failure of an antioxidant system. It is assumed that the high antioxidant state by CuONPs (1, 2, and 5 mg/kg b.wt) protects against lipid peroxide by scavenging free radicals. From these results, we conclude that the CuONPs positively modify the state of antioxidants and restore them to an almost regular rate

(Table 3). Our findings are consistent with Zhang et al. [71], who have shown that AgNPs have a positive impact on the liver in terms of lipid peroxides. Administration with AgNPs was effective in relieving CCl₄ injuries [74]. Mohanta et al. [75] showed that biosynthesized AgNPs have potent antioxidant activities, as they have a protective impact on free radical generation or inhibit their production.

3.8. The Effect of Copper Oxide Nanoparticles Produced Using *Streptomyces* sp. MHM38 against Paracetamol-Induced Liver Tissue Damage. The results of the histopathological examination conducted in this study showed that the average group exhibited a standard lobular structure and normal hepatic cells (Figure 12(a)). Simultaneously, significant vacuolar degeneration of hepatocytes and fibroplasia occurred in the portal triad in the paracetamol-treated group (Figure 12(b)). These results are consistent with Madkour and Abdel-Daim [73], who explained that paracetamol caused inflammatory necrosis in liver tissue. Treatment with CuONPs reduced the pathological changes of paracetamol, which enhanced its ability to protect the liver from paracetamol toxicity (Figures 12(f)–12(h)). These results are consistent with Keshari et al. [76]. Bhuvaneshwari et al. [77] showed that nanoparticle ameliorates liver tissue for CCl₄-treated rats. The drug's ability to reduce injuries or maintain physiological liver function after induction of poisoning indicates its hepatoprotective impact [78].

4. Conclusions

Biosynthesis of CuONPs using actinomycetes is an emerging trend in bio-nanotechnology and thus has a broad range of implementation in the biomedical field due to its eco-friendly nature and biocompatibility. The extensive function of *Streptomyces* sp. MHM38 is very useful for generating CuONPs in a nontoxic manner. The biosynthesized CuONPs were characterized by UV-vis spectroscopy, XRD, EDX, and TEM analysis. The formed CuONPs showed a prominent antimicrobial activity at different concentrations against the pathogens, viz., *Candida albicans* ATCC 10237, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 8939, *Pseudomonas aeruginosa* ATCC 9027, and *Enterococcus faecalis* ATCC 29212. In addition, CuONPs biosynthesized from marine *Streptomyces* sp. MHM38 had no toxic effect on the liver of the studied rats. It also mitigated the adverse impact of paracetamol, indicating that it can be used for several beneficial purposes, including as a prophylactic, without implications for the liver. Finally, this research opens up new ways to explore the role of biologically synthesized nanostructured substances for multi-action use in medical purposes.

Data Availability

Data are available on request. Please contact Mohamed Alagamy.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Moaz M.H., Hanaa S.S.G., Asmaa M.Y., Sarah I.B., and Mohamed H.A. performed the methodology. Moaz M.H. was responsible for the software. Moaz M.H., Hanaa S.S.G., and Asmaa M.Y. contributed in writing. Mohamed H.A. contributed in data curation, writing, review, and editing. Hesham H.R. was responsible for the supervision. All authors read and approved the final manuscript.

Acknowledgments

The authors extend their appreciation to the Deputyship for Research & Innovation, "Ministry of Education" in Saudi Arabia, for funding this research work through the project no. IFKSURG-038.

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