Biosynthesis of Copper Oxide Nanoparticles by Marine Streptomyces MHM38 and their Preventive Efficacy Against Paracetamol-Inducing Hepatic Damage of Albino Rats

Moaz Hamed (Moaz-micro@hotmail.com)  
National Institute of Oceanography and Fisheries Mediterranean Sea Branch  
https://orcid.org/0000-0002-2608-085X

Hanaa S.S. Gazwi  
Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, El-Minia, Egypt

Asmaa M. Youssif  
Department of Botany and Microbiology, Faculty of Science, Alexandria University

Research

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Abstract

Biosynthesis methods, employing microorganisms, have emerged as an eco-friendly, clean and viable alternative to chemical and physical methods. The present study reports the biosynthesis of copper oxide nanoparticles (CuONPs) using cell-free culture supernatant of marine Streptomyces sp. MHM38. For the optimized production of copper oxide nanoparticles, the influence of some parameters such as concentration of copper sulphate, reaction time, filtrate to substrate ratio and pH were studied. 5 mM CuSO4 was optimal for NP production. Well-defined CuONP formation occurred after 60 min incubation when equal volume of filtrate (cell-free supernatant) to substrate (CuSO4 solution) was added. NPs remained stable in aqueous solution with increasing time at pH 8. CuONPs were characterized by UV-vis spectroscopy, X-ray diffraction (XRD) and finally the nature of the nanoparticles was identified by elemental analysis (EDX). UV-vis spectroscopy of CuONPs exhibited peak at 550 nm which corresponds to the Surface Plasmon Resonance of CuONPs. Most of the particles are spherical in shape and size ranges from 1.06 – 6.5 nm analyzed using Transmission Electron Microscope (TEM). Antimicrobial activity of CuONPs was performed by 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, Aspergillus niger) and yeast (Candida albicans ATCC 10237). The highest antimicrobial activities recorded were against Candida albicans ATCC 10237, were as Salmonella typhimurium ATCC 14028 and Escherichia coli ATCC 8939 showed the lesser activity. The preventive efficacy of CuONPs was evaluated against the oxidative stress induced by paracetamol (PAC) in albino rats. The biochemical findings of CuONPs groups appeared a significant (p<0.05) diminish in the levels of ALT, AST, ALP, LDH, total and direct bilirubin, Urea, and Creatinine as compared to paracetamol group. Non-enzymatic and enzymatic antioxidants of CuONPs groups were significantly elevated (p<0.05) in SOD and GSH levels and significantly low NO and MAD levels compared to the paracetamol group. Also, the histopathological examination of the CuONPs groups assured that the impact of improving CuONPs against paracetamol-induced liver damage.

Introduction

Because of their special electrical, optical, catalytic and magnetic characteristics, metal nanoparticles are synthesized and used (Zhang et al., 2016; Jeevanandam et al., 2018; Khan et al., 2019) which are different from bulk materials. Many chemical and physical methods have recently demonstrated the synthesis of inorganic nanoparticles. Nevertheless, the value of biological synthesis is realized globally as dangerous, expensive and non-environment-friendly chemical methods (Shantkriti and Rani, 2014). It is thus important to develop fast, cost-effective, ecological and easy-to-scale synthetic approaches. The development of green roads to manufacture nanoparticles of metal using biological systems is therefore important. It has been well established that microbes develop mechanisms to survive in toxic metals by tuning toxic metal ions into their corresponding nontoxic forms as metal sulfide / oxides, in harsh conditions (Tenkouano, 2017). The specifics of nano-transformation mechanisms are not well known. For nanoparticles synthesis, a wide range of biological tools can be used such as bacteria, yeasts, fungi, algae and plants. A large number of the world’s population uses nanoparticles to treat some diseases. Recent studies have indicated that CuNPs have broad biological properties (Murthy, 2007).

Paracetamol is a medication with antipyretic and analgesic impacts that is broadly utilized by the wider community. Paracetamol is taken freely without supervision, however high doses of paracetamol cause liver damage. Paracetamol is expected to be a major factor in the cause of severe liver damage (Nerdy and Ritarwan, 2019). This present study has been designed to use marine actinobacterial strains namely, Streptomyces sp. that was screened for biosynthesis, optimization and characterization of CuNPs produced as well as to evaluate their inhibitory action against some pathogenic microorganisms and conducted as an attempt to see if animal tissue accepts CuONPs and whether CuONPs have antioxidant and hepatoprotective activity.

Material And Methods

Microorganisms and cultural conditions

Streptomyces sp. MHM38 was isolated by Dr. Moaz M. Hamed (Marine Microbiology Lab., Marine Environmental Division, National Institute of Oceanography and Fisheries, Egypt) from the marine sediment sample in Suez gulf, and deposited in the Genbank as Streptomyces sp. MHM38 with accession number KU764745. This marine actinobacterial isolate was maintained on slopes containing starch nitrate agar medium (SNM) of the following composition (g/l): Starch, 20; K2HPO4, 1; KNO3, 2; MgSO4, 0.5; agar, 18. Components were dissolved in 0.5-liter distilled water and 0.5-liter seawater (El-Sersy et al., 2010). The 50 and 20 μg mL-1 tetracycline and nystatin as antibacterial and anti-fungal agents to prevent bacterial and fungal infection were applied following autoclaving and solidification. Strain was incubated for a period of 7 days at 30-32°C. The isolate was stored as spore suspension in 20% (v/v) glycerol at -20°C for subsequent investigation.

Inoculum preparation

250 mL Erlenmeyer flask containing 50ml of medium consisting of (g/l): Starch, 20; K2HPO4, 1; KNO3, 2; MgSO4, 0.5. Components were dissolved in 0.5 liter distilled water and 0.5 liter seawater. This flask was inoculated with old stock culture grown on starch nitrate agar medium. The flask was incubated for 5 days in a rotator incubator shaker at 30-32°C, 200 rpm, and was used as inoculums for subsequent experiments.

Extracellular synthesis of CuONPs

In order to screen Streptomyces sp. MHM38 for the synthesis of CuONPs, the isolate was freshly inoculated in an Erlenmeyer flask containing 50ml of the production medium consisting of (g/l): Starch, 20; K2HPO4, 1; KNO3, 2; MgSO4, 0.5. Components were dissolved in 0.5 liter distilled water and 0.5 liter
seawater. The inoculated ask was incubated for 5 days in a rotator incubator shaker at 30-32°C and 200 rpm. The culture was centrifuged at 10,000 rpm at the end of the incubation, and supernatants were used to detect copper nanoparticles. A volume of 15 ml of 1mM CuSO₄ was added to 15 ml of each supernatant in 100 ml Erlenmeyer flasks. Flasks were incubated at 30-32°C and observed for color change. Two controls were used, first control (sterile media mixed with 1mM Copper sulphate) to establish that media components cannot reduce the copper ions to copper nanoparticles and a negative control (copper sulphate solution) to confirm that no color change is observed by time. For any visual color change the flasks were observed daily. Usage of UV-visible spectroscopy in the range 200-800 nm was measured for flasks with color adjustment (Shantkriti and Rani, 2014).

Optimization of different factors on the production of CuONPs by Streptomyces sp. MHM38

Effect of copper concentration on NPs production

In 15 mL of 1mM to 10 mM CuSO₄, 15 mL of cell-free supernatant were added. The blend was incubated as above and UV-vis-spectroscopy was used for the collection and study of CuONPs.

Effect of reaction time on NPs production

Due to time, nanoparticles synthesis and stability are significant. 15 mL of cell-free supernatant was added to 15 mL of 5mM CuSO₄ solution. The mixture has been incubated for various periods as above; for CuONPs, a UV vis analysis has been performed and analyzed.

Effect of substrate to filtrate ratio on NPs production

In order to study the effect of different substrate to filtrate ratio on the CuONPs formation, three flasks were prepared one contained 15 mL of cell-free supernatant was added to 15 mL of 5mM CuSO₄ solution; other one 15 mL of cell-free supernatant was added to 7.5 mL of 5mM CuSO₄ solution and the last one 15 mL of cell-free supernatant was added to 30 mL of 5mM CuSO₄ solution. The mixture was incubated static for 60 min and CuONPs formed were analyzed by UV-vis spectroscopy.

Effect of pH on NPs production

Cell-free supernatant was exposed to 5 mm CuSO₄ at pH 6, 7 and 8 and incubated for 60 min in order to investigate the impact of pH on CuNPs production. UV-vis spectroscopy has analyzed the formed CuONPs.

Characterization of copper nanoparticles

UV-visible spectral analysis

Color changes were observed for biosynthesized copper nanoparticles using the free cell supernatant. CuNPs was characterized by UV-visible spectroscopy in range of 200-800 nm, at regular intervals.

Transmission Electron Microscope analysis

CuONPs solution was diluted and sonicated. A drop was placed on carbon coated grid and water evaporated. TEM measurements were performed on a JEM-100-CX, worked at a voltage acceleration of 80 KV dry, then examined with a transmission electron microscope at the Faculty of Science, Alexandria University.

Energy Dispersive X-ray (EDX) spectroscopy analysis

The technique described by (Jyoti et al., 2016) is used mainly to determine the elementary structure of the sample and to confirm that the suspension of the nano part contained only copper (Roy et al., 2013). This analysis was done by using powder of lyophilized copper nanoparticles. A sample was analyzed using Oxford instrument attached to scanning electron microscope at the Electron Microscope Unit, Faculty of Science, Alexandria University.

X-ray Diffraction (XRD) analysis

CuONPs sample was dried for XRD pattern analysis recorded in the transmission mode on Shimadzu XRD7000 instrument (at the Central Laboratory, City of Scientific Research and Technological Applications, Egypt) operating at 40KV current 30mA with Cu Ka radiation (λ=1.5404 Å) (Fayaz et al., 2011). A monochromatic X-ray beam with wave length lambda was used to analyze the crystalline nature of the sample (Karthik et al., 2014)

Biotechnological application of copper nanoparticles

Antimicrobial activity of CuNPs by agar diffusion method

Biosynthesized Cu-NPs 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, Escherichia coli ATCC 8939), fungi (Rhizoctonia solani, Fusarium solani, Aspergillus niger) and yeast (Candida albicans ATCC 10237) using the well-diffusion method on Mueller-Hinton Agar (MHA) was used for bacteria, Sabouraud Dextrose Agar (SDA) was used for C. albicans and potato D-glucose agar (PDA) used for fungi. A 100 μl bacterial suspension was used in the preparation of bacterial lawns for each bacterial test organism. The staff of the National Institute of Oceanography and Fisheries (NIOF).
Alexandria branch received all the bacterial and yeasts cultures. The Assiut Mycological Center (AUMC) University, Assiut, Egypt, supplied fungal cultures as well. An 8mm diameter agar well was made by a sterilized cork borer in stainless steel. The wells were loaded with 100 μl of 200 mg/mL concentrations of CuO nanoparticles solution and 100 μl of culture broth from Streptomyces sp. MHM38 cell free supernatant and a DMSO was used as a solvent. The plates were incubated for 24 hours at 37°C for bacteria and 120 hours at 28°C for fungi then examined for inhibition zones. The diameter of such inhibition areas was measured and the mean value was recorded in millimeters for each organism (El-Naggar and Abdelwahed, 2014).

CuO NPs against oxidative stress

Animal and Experimental Design

Eighty healthy, 8-week-male albino rats (Sprague – Dawley strain) weighing 180–200g was obtained and housed in the biology lab in Agricultural Chemistry Department, Faculty of Agriculture, Minia University at a controlled temperature of (25 ± 2°C) with 12 hr. 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 (10 rats in each group); and subjects for 21 days to one of the following treatments:

Group 1: Normal
Group 2: Administered with Paracetamol (PAC) (500 mg/kg b. wt) orally daily for the last 5 days (Al-Rubaei et al., 2014)
Group 3: Administered with CuO NPs (1 mg/kg b. wt) orally daily for 21 days
Group 4: Administered with CuO NPs (2 mg/kg b.wt) orally daily for 21 days
Group 5: Administered with CuO NPs (5 mg/kg b.wt) orally daily for 21 days
Group 6: administered with CuO NPs (1 mg/kg b. wt) alone for 16 days and then received both CuO NPs and paracetamol in the last 5 days
Group 7: Administered with CuO NPs (2 mg/kg b. wt) alone for 16 days and then received both CuO NPs and Paracetamol in the last 5 days
Group 8: Administered with CuO NPs (5 mg/kg b. wt) alone for 16 days and then received both CuO NPs and paracetamol in the last 5 days

After 24 hours, rats were sacrificed under light ether narcosis followed by decapitation 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 minutes to separate the serum. The serum obtained was used to estimate biochemical analyses following: alanine aminotransferase (ALT), aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) , alkaline phosphatase (ALP), total and direct bilirubin, urea, and creatinine which were measured using by methods (Bessey et al., 1946; Reitman and Frankel, 1957; Weatherburn, 1987; Hanan and Riham, 2012; Mohamed et al., 2019) and (Koštíř and Šonka, 1952), respectively.

Homogenization of the liver was done in homogenization buffer PBS (pH 7.4) and then homogenates were centrifuged at 10,000 g for 30 min (+4 °C) to obtain supernatants. The supernatants of liver tissues of rats were used to analyze for glutathione (GSH) levels according to (Davies et al., 1984), nitric oxide (NO) according to (Montgomery and Dymock, 1961), malondialdehyde (MDA) according to (Ohkawa et al., 1979), and superoxide dismutase (SOD) according to (Kakkar et al., 1984).

Liver tissues were also fixed in neutral buffered formalin10 %, 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 (H&E), and Prussian blue reaction according to (Bancroft and Gamble, 2008)

Statistical analysis

Data are presented as mean ± standard error of the means (SEM). One-way analysis of variance (ANOVA) followed by Tukey post-hoc test using the Statistical Package for the Social Sciences (SPSS software V. 18.0) were performed for statistical analysis. P values < 0.05 were considered as the significant level.

Results And Discussion

Evaluation of biosynthesis of Copper Oxide nanoparticles by Streptomyces sp.MHM38

Streptomyces sp. MHM38 had the potential to reduce the copper ions to copper nanoparticles. The biosynthesis of copper nanoparticles was indicated by changing the faint blue reaction blend to green after 1 % (v / v) of a 1 mM aqueous CuSO₄ was added to the cell-free Streptomyces sp. MHM38 supernatant. No color change, by comparison, was observed in aqueous CuSO₄ incubated under the same conditions without free cell supernatant (Fig.1). The formation of colors depends on the surface vibration of Plasmon on the surface (Shantkriti and Rani, 2014). Our results agree with (Shantkriti and Rani, 2014) who mentioned that when cell-free supernatant of Pseudomonasfluorescens was added to CuSO₄ solution and incubated for 48 h, the reaction mixtures color changed from blue to dark green.

UV-Visible spectral analysis
The presence of nanoparticles with the UV-visible spectrophotometry within the 200-800 nm range has been confirmed. The copper nanoparticles formed by Streptomyces sp. MHM38 showed an absorption peak of 550 nm (fig. 2) of the different Surface Plasmon Resonance spectra (SPR), indicating the existence of CUNPs. Depending on individual particle properties like size, shape and capping agents, the exact location of the SPR Band can vary (Mott et al., 2007). (Brause et al., 2002) reported that surface-plasmon resonance is dominant in the optical absorption of metal nanoparticles and particle size is linked to the absorption peak. Copper nanoparticles in aqueous solution have the Surface Plasmon Resonance increase to longer wavelengths, with particle size increasing. The position and form of copper nanocluster absorption of plasmon is strongly dependent on the particle size, stabilizing molecules or surface adsorbed particles, and bioelectricity of the media. (Krishnaraj et al., 2010). Our results agree with those of (Hamid, 2015) who mentioned that the copper Surface Plasmon Resonance (SPR) band of Salmonella typhimurium occurred at 565 nm. (Shantkriti and Rani, 2014) who mentioned that the copper Surface Plasmon Resonance (SPR) band of Pseudomonas fluorescens exhibits a distinct absorption peak in the region of 550-650 nm.

**Optimization of CuONPs by Box-Behnkin design**

**Effect of copper concentration on NPs production**

From Fig. 3, it is clear that the rate of formation for CuONPs increased with the increase of substrate concentration reaches its maximum at 5 mM, as it was shown here the best observation for CuONPs is at concentration 5mM of CuSO₄. Addition of various concentrations of CuSO₄ solution to pellet did not reveal any color change. Our results agree with those of (Shantkriti and Rani, 2014) who mentioned that reaction mixtures color changed from blue to dark green after addition of 5mM CuSO₂ to supernatant of Pseudomonas fluorescens. A close look to a dark green solution with 5 mM CuSO₄ applied to a flask containing Morganella sp. (Ramanathan et al., 2011).

**Effect of reaction time on CuONPs production**

The synthetizations and stability of nanoparticles has been an important element since time. The absorption at 550 nm was shown to progressively increase till 60 minutes, so there was no change. This suggests the development of CuONPs and the reduction of the size occurs with growing time. While Kimber R. et al., (2018), who mentioned that complete reduction of CuSO₄ solution to CuNPs by Shewanella oneidensis after 96 h and (Hamid Reza Ghorbani, 2015) who mentioned that the CuNPs of Salmonella typhimurium formed after 20 min. On the other hand, (Shantkriti and Rani, 2014), CuONPs formed by Pseudomonas fluorescens after 90 min after addition of 5 mM CuSO₄ solution.

**Effect of substrate to filtrate ratio on CuONPs production**

Different volumes of CuSO₄ solutions have been used to understand CuSO₄ volume required for the efficient production of NPs. When samples were taken at 60 min with a ratio of filtrate (cell-free supernatant) to substrate (CuSO₄ solution) was 1:1, absorbance of CuONPs at 550nm gave high value in comparison with ratio of filtrate to substrate (cell-free supernatant) to substrate (CuSO₄ solution) was 1:1/2 or ratio of filtrate to substrate (CuSO₄ solution) was 1:2 as shown in Fig. 5.

**Effect of pH on CuONPs production**

Altering the pH is said to help control nanoparticles’ shape and size (Gurunathan et al., 2009). The peaks of acidic pH of 6 were not typical of CuONPs. The alkaline pH has provided a high absorption peak at 550 nm. While (Shantkriti and Rani, 2014), characteristic peak of CuONPs formed by Pseudomonas fluorescens at neutral pH. After optimization we can estimate the most optimum conditions for CuONPs was substrate concentration 5mM, equal volume of filtrate and substrate were added, pH adjusted at 8 and incubated static for 60 min. As showed in Fig.7, the color of solution changed to green color when we applied the preview conditions, which indicates high production of CuONPs.

**Characterization of CuONPs**

**Transmission Electron Microscope (TEM)**

Many studies have classified copper nanoparticles in their shape and size based on TEM structures (Singh et al., 2015). The present work reveals the spherical form of nanoparticles in the TEM images of copper nanoparticles (Fig. 8). TEM analysis of CuONPs produced by Streptomyces sp. MHM38 are relatively uniform in shape. In general, spherical particles appear with an average dimensional size of 1.09 – 6.54 nm. These are smaller than CuNPs formed by Pseudomonas fluorescens showing size of 20–80 nm (Shantkriti and Rani, 2014). Kimber R. et al., (2018), mentioned that Shewanella oneidensis produced spherical CuNPs in the range of 20–50 nm.

**X-ray diffraction (XRD) analysis**

The XRD pattern of nanoparticles showed intensive peaks throughout the 20° range of the value 20–80, similar to the copper-oxide nanoparticle reflection of the Bragg's. Thus, the reaction mixture indicated the formation of copper oxide nanoparticles. CuONPs produced by Streptomyces sp. MHM38 distinguished XRD peaks with 2θ values of distinguished XRD peaks with 2θ values of 35°, 38°, 48°, 53°, 58°, 65°, 67° & 72° were observed (Fig.9). These peaks are assigned to the (111), (202), (020), (202) & (113) reflection planes of face-centered-cubic (fcc) copper, respectively.

Our results agree with those of (Chen et al., 2017) who mentioned that the X-ray diffraction pattern of the copper nanoparticles synthesized by N,N'-dicab oxy methyl perylene diimide (PDI) functionalized CuO nanocomposites showed at 2θ values of 35°, 38°, 48°, 53°, 58°, 65°, 67° & 72° corresponding to
XRD planes (111), (202), (020), (202) & (113) Bragg's reflection based on the fcc structure of CuONPs.

**Energy dispersive X-ray (EDX) spectroscopy analysis**

EDX and element mapping determined the purity and elemental composition of the nanoparticles. In the current research EDX spectroscopy analysis was performed for CuONPs produced by *Streptomyces* sp. MHM38 (Fig.10), which confirmed the presence of elemental copper by the signals. In the EDX spectrum, the nanoparticles displayed a peak at 8 keV, which is due to the absorption of copper oxide nano crystallites corresponding to surface plasmon resonance (Maqbool et al., 2018). The optical absorption band peak for nanoparticles produced by *Streptomyces* sp. MHM38 was in the range of 1 to 9 keV is typical for the absorption of copper oxide nano crystallites. The main component observed was copper oxide; other elements were observed, such as calcium, chloride, phosphate and carbon. The carbon distribution was triggered by the use of a TEM network. However, there were other EDX peaks for calcium, phosphorus and chloride and that also represents an essential ingredient in bacterial structural proteins that have functional groups suggesting that they were mixed precipitates from the centrifuged supernatant/metal solution.

**Antimicrobial activity of biosynthesized CuONPs**

Nanoparticles have elevated surface-volume ratio, tiny size and elevated dispersion characteristics that enable them to interact with microbial surfaces. The Cu-NPLs’ large surface area enhances their interaction with the microbes in order to perform wide antimicrobial operations (Shende et al., 2015). However, the few reports on Cu-NPLs antimicrobial research have shown that Cu-NPLs are effective against multiple pathogenic microorganisms (Hassanien et al., 2018). The anti-microbial activity of CuONPs was carried out on pathogenic bacterial strains Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212), Gram-negative bacteria (*Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8939), fungi (*Rhizoctonia solani*, *Fusarium solani*, *Aspergillus niger*) and yeast (*Candida albicans* ATCC 10237) using the method of well diffusion, and the inhibition area values are shown in (Table 1 & figure 11). In each plate and DEMSO as control, cell-free supernatant broth with no CuSO4 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. Such findings are consistent with those of previous studies which examined *Candida albicans* and *Pseudomonas aeruginosa* antimicrobial activity of Cu-NPs. (Hassanien et al., 2018). The appearance of the inhibition area showed that in these locations there was no growth of bacteria. This shows how biosynthesized Cu-NPs interact with a smaller part / high surface area, of which Cu-NPs have been adsorbed onto the surface of the microorganism cell wall. As a result of this, cell walls that destroy human pathogens were demolished and disrupted by the resistance property of biosynthesized Cu-NPs. The antibacterial activity of copper metal is licensed by the United States as a microbial. EPA Agency (Environmental Protection Agency) (Hassan et al., 2019). The inhibitory action of Cu-ONPs can be due to their small size and high volume-to-surface area, allowing it to interact with microbial cell membrane (Usman et al., 2013). In addition, 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 inactivating important enzymes (Yoon et al., 2007). Espirito Santo et al. indicated that Cu-NPs inhibitory action associated with inactivated surface protein responsible for transporting material across cytoplasmic membranes and destroying selective permeability (Santo et al., 2008). Marine actinomycetes have recently revealed biosynthesis of CuONPs and their applications against pathogenic microbes (Rasool and Hemalatha, 2017).

**The effect of CuONPs produced by *Streptomyces* sp. MHM38 against paracetamol-induced liver and kidney damage**

The tests for ALT, AST, ALP, and bilirubin are important 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, there is an expansion in these two parameters. LDH is a conspicuous marker and a diagnostic tool for tissue injury. As shown in the Table 2, there were no significant differences in ALT, AST, ALP, LDH, total and direct bilirubin, Urea, and Creatinine between the groups treated with Cu-NPs compared with the control group. Although these parameters increased with increasing dose of Cu-ONPs. Conversely, significant increases were demonstrated in the ALT, AST, ALP, and LDH, total and direct bilirubin, Urea, and Creatinine levels in paracetamol treated rats compared to 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., 2013) who explained that paracetamol increased levels of ALT, AST, ALP, and bilirubin, Urea, and Creatinine. Paracetamol turns into water-soluble products by drug metabolism enzymes and excreted in the urine in a therapeutic dose (Hinson et al., 2010). Excess paracetamol is oxidized to N-acetyl-pbenzoquinone imine (NAPQI) which is a toxic and is done by the hepatic cytochrome p450 system (CYP450) (Yen et al., 2007). Detoxification is normally expelled from NAPQI by GSH. GSH depletion occurs when using high doses of paracetamol and consequently the toxic NAPQI accumulation that binds to cellular proteins via cysteinyl sulfhydryl groups and forms NAPQI-protein adducts (Subramanian et al., 2013). This case results in the formation of reactive oxygen species (ROS) like superoxide anion (O2^•−), hydrogen peroxide (H2O2), and a hydroxyl root (OH•) that impacts the cell membrane and stimulates lipid peroxide and also leads to liver necrosis (Chen et al., 2009). Liver cell injuries lead to cellular enzymes to leak into the bloodstream and can, therefore, 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 (Moshiafe-Nezhad et al., 2019). (Farghaly and Hussein, 2010) reported that Paracetamol caused an increase in the level of LDH. Paracetamol works to accumulate Ca^{2+} intracellular, which activates anaerobic glycolysis and phosphofructokinase, forming lactate and thus increasing LDH (Landowne and Ritchie, 1971). Returning these enzymes to normal levels demonstrates the protective effect of CuO-NPs against liver and kidney damage and their ability to regenerate liver and kidney cells. These results agree with (Zhang et al., 2019). Green synthesized nanoparticles exhibit a beneficial effect against liver and kidney degradation because the bacteria used in the synthesis of nanoparticles have medicinal properties. These results are consistent with (Ghaffar et al., 2014). The protective activity of CuO-NPs may be attributed to its role in preventing cellular leakage and losing the functional integrity of the cellular membrane in hepatocytes and kidney.
The effect of CuO-NPs produced by *Streptomyces* sp. MHM38 against paracetamol-induced oxidative stress

In this study, the oral administration of biosynthesized CuO-NPs alone did not induce any obvious changes in the most biochemical parameters compared with the control group. The administration of paracetamol produced a significant increase in NO and MAD content accompanied by a marked inhibition of GSH and SOD activities compared with the control group. The administration of biosynthesized CuO-NPs led to a significant decrease in NO and MAD content and an increase of GSH and SOD levels compared to the paracetamol group (Table 3).

Non-enzymatic antioxidants (GSH) and enzymatic antioxidants (SOD) in natural conditions regulate the removal and production of free radicals and thus maintain the level of ROS. Thus, this antioxidant protects the body from oxidative stress. (Ravindran et al., 2013) 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, 2013) 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 are unable to remove them and prevent their formation. An increased level of MDA and NO and decreased level of SOD and GSH is an indication of tissue damage and failure of an antioxidant system.

It is assumed that the high antioxidant state by CuO-NPs (1, 2 and 5 mg/kg.b.wt) provides protection against lipid peroxide by scavenging free radicals. From these results, we conclude that the CuO-NPs positively modify the state of the antioxidants and restore them to an almost normal rate (Table 3). Our findings are consistent with (Zhang et al., 2019) who have shown that AgNPs have a positive impact on the liver in relation to lipid peroxides. Administration with AgNPs was effective in relieving CCl₄ injuries (D’Antoni, 2016). (Mohanta et al., 2017) appeared that Biosynthesized AgNPs have strong antioxidant activities as they have a protective impact on free radical generation or inhibit their production.

The effect of CuO-NPs produced by *Streptomyces* sp. MHM38 against paracetamol-induced liver tissue damage

The results of the histopathological examination conducted in this study showed that the normal group exhibited normal lobular structure and normal hepatic cells (Figure 12 (A). While significant occurred vacuolar degeneration of hepatocytes and fibroplasia in the portal triad in the paracetamol-treated group (Figure 11(b). These results are consistent with (Madkour and Abdel-Daim, 2013) who explained that Paracetamol caused inflammatory necrosis in liver tissue.

Treatment with CuO-NPs reduced the pathological changes caused by paracetamol, which enhanced its ability to protect the liver from paracetamol toxicity (Figures 11(F, G and H). These results are consistent with (Keshari et al., 2018). (Bhuvaneswari et al., 2014) appeared that nanoparticle ameliorates liver tissue for CCl4-treated rats. The drug’s ability to reduce injuries or maintain the physiological liver function after induction of poisoning is an indication of its hepatoprotective impact (Yadav and Dixit, 2003).

Conclusions

The results appeared that copper nanoparticles biosynthesized from marine *Streptomyces* sp. MHM38 has no toxic effect on the liver of the studied rats; it also mitigated the adverse effects of paracetamol, however. This shows that it can be used for several beneficial purposes, including prophylactic impacts on the liver.

Declarations

Ethics approval and consent to participate

Approval

Consent for publication

We are agree

Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Competing interests

There are no conflicts of interest

Funding

Nil

Authors’ contributions

Dr/Moaz, Dr/Hanna and Dr/ Asmaa contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.
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Tables

Table 1: Antimicrobial activity of copper nanoparticles produced by Streptomyces sp. MHM38

| Microorganism                          | Inhibition zone diameter (mm) | Free supernatant | CuONPs | DEMSO |
|----------------------------------------|------------------------------|------------------|--------|-------|
| *Enterococcus faecalis* ATCC 29212     | 0.0                          | 20.0             | 0.0    |       |
| *Salmonella typhimurium* ATCC 14028    | 0.0                          | 18.0             | 0.0    |       |
| *Pseudomonas aeruginosa* ATCC 9027     | 16.0                         | 20.0             | 0.0    |       |
| *Escherichia coli* ATCC 8939           | 0.0                          | 18.0             | 0.0    |       |
| *Rhizoctonia solani*                   | 0.0                          | 0.0              | 0.0    |       |
| *Fusarium solani*                      | 0.0                          | 0.0              | 0.0    |       |
| *Aspergillus niger*                    | 0.0                          | 0.0              | 0.0    |       |
| *Candida albicans* ATCC 10237          | 18.0                         | 22.0             | 0.0    |       |

Table 2: Effect of CuO-NPs produced by Streptomyces sp. MHM38 on biochemical parameters

| Groups | Control | PAC | CuO-NPs (1 mg/kg b.wt) | CuO-NPs (2 mg/kg b.wt) | CuO-NPs (5 mg/kg b.wt) | CuO-NPs (1 mg/kg b.wt) + PAC | CuO-NPs (2 mg/kg b.wt) + PAC | CuO-NPs (5 mg/kg b.wt) + PAC |
|--------|---------|-----|------------------------|------------------------|------------------------|-----------------------------|-----------------------------|-----------------------------|
| ALT (U/l) | 22.53±0.59 | 82.90±3.06 | 25.60±0.78 | 24.40±1.21 | 26.35±0.64 | 42.23±1.88 | 39.50±0.89 | 52.37±2.42 |
| AST (U/l) | 80.80±1.73 | 223.65±7.59 | 82.48±6.15 | 73.65±1.99 | 85.17±1.85 | 136.25±2.28 | 109.65±0.43 | 153.9±0.84 |
| ALP (U/l) | 223.2±3.67 | 611.1±5.77 | 229.4±1.09 | 226.95±3.95 | 231.8±7.63 | 338.67±5.27 | 360.03±25.12 | 411.8±9.61 |
| Total bilirubin (mg/dl) | 0.65±0.03 | 2.27±0.09 | 0.75±0.03 | 0.7±0.06 | 0.8±0.06 | 1.23±0.13 | 0.95±0.03 | 1.7±0.06 |
| Direct bilirubin (mg/dl) | 0.4±0.06 | 0.99±0.06 | 0.4±0.12 | 0.38±0.18 | 0.45±0.02 | 0.58±0.02 | 0.51±0.2 | 0.7±0.05 |
| Urea (mg/dl) | 23.45±1.59 | 50.97±1.42 | 24.5±1.10 | 23.83±0.80 | 27.13±0.57 | 33.2±0.35 | 31.70±0.23 | 40.15±0.02 |
| Creatinine (mg/dl) | 1.25±0.03 | 2.85±0.03 | 1.31±0.03 | 1.30±0.06 | 1.35±0.03 | 1.70±0.06 | 1.58±0.03 | 1.9±0.11 |
| LDH (U/l) | 145.7±0.14 | 374.4±7.04 | 155.7±2.59 | 146.0±1.73 | 158.25±0.14 | 262.85±1.59 | 235.7±2.59 | 283.5±2.02 |

Results are expressed as mean ± SE (n=10) where mean significant at p < 0.05. *compared with normal group; **compared with paracetamol group.
Table 3: Effect of CuO-NPs produced by Streptomyces sp. MHM38 on NO, MAD, SOD, and GSH

| Groups | Control | PAC | CuO-NPs 1 mg/kg b.wt | CuO-NPs 2 mg/kg b.wt | CuO-NPs 5 mg/kg b.wt | CuO-NPs (1 mg/kg b.wt) +PAC | CuO-NPs (2 mg/kg b.wt) +PAC | CuO-NPs (5 mg/kg b.wt) +PAC |
|--------|---------|-----|----------------------|----------------------|----------------------|----------------------------|----------------------------|----------------------------|
| NO (umol/g tissue) | 46.92±0.15 | 66.65±1.69 | 51.67b±0.07 | 51.27b±0.14 | 51.17b±1.48 | 60.35ab±1.68 | 54.58ab±1.57 | 61.49ab±1.94 |
| MDA (nmol/g tissue) | 8.15±0.53 | 24.40a±0.23 | 9.40b±0.46 | 8.60b±0.40 | 9.50b±0.25 | 12.27ab±0.38 | 15.10ab±0.06 | 17.19ab±1.88 |
| SOD (U/g protein) | 23.58±2.00 | 9.84a±1.54 | 21.34b±0.64 | 23.61b±0.59 | 23.05b±1.69 | 12.65ab±0.37 | 19.41ab±1.49 | 17.13ab±1.38 |
| GSH (nmol/g tissue) | 4.45±0.17 | 1.54a±0.02 | 4.46b±0.03 | 4.02b±0.01 | 4.18b±0.33 | 3.50ab±0.23 | 3.00ab±0.1 | 2.92ab±0.01 |

Results are expressed as mean ± SE (n=10) where mean significant at p < 0.05. a compared with normal group; b compared with paracetamol groups.

Figures

Figure 1

Visible observation of CuONPs biosynthesis by Streptomyces sp. MHM38 (left) Cell free supernatant of 24h old culture (15 ml) was added to 15 ml of 1mM CuSO4 solution and incubated static for 5 days, (Right) un-inoculated medium (15 ml) was added to 15 ml of 1mM CuSO4 solution and incubated static for 5 days.
Figure 2

UV-Vis absorption spectrum of copper nanoparticles synthesized by cell free supernatant of *Streptomyces* sp. MHM38 supernatant of 72h old culture (15 ml) was added to 15 ml of 1mM CuSO4 solution and incubated static.

Figure 3

Effect of different CuSO4 concentrations on the production of CuONPs by *Streptomyces* sp. MHM38.
Figure 4

Effect of reaction time on the production of CuONPs by Streptomyces sp. MHM38.

Figure 5

Effect of ratio between filtrate (cell-free supernatant) to substrate (CuSO4 solution) concentration on the production of CuONPs by Streptomyces sp. MHM38.

Figure 6

Effect of pH on the production of CuONPs by Streptomyces sp. MHM38.
Figure 7

Visible observation of optimized CuONPs biosynthesis by Streptomyces sp. (Left: 15 ml of supernatent was added to 15 ml of 5mM CuSO4 at pH 8. Right: 15 ml of media was added to 15 ml of 5mM CuSO4 at pH8).

Figure 8

TEM image of produced spherical CuONPs using cell-free supernatant of Streptomyces sp. MHM38.
Figure 9

Shows X-Ray diffraction pattern with regards to the biosynthesized CuONPs by Streptomyces sp. MHM38.

Figure 10

EDX spectrum of CuONPs formed by Streptomyces sp. MHM38 showing peak between 1, 8 and 9 KeV.
Figure 11

Antibacterial activity of Cu-NPLs against Candida albicans ATCC 10237, Escherichia coli ATCC 8939 and Pseudomonas aeruginosa ATCC 9027 (A: Cell-free supernatant, B: copper nanoparticles produced by Streptomyces sp. MHM38 and C: DEMSO).

Figure 12

Section of livers rats.

Supplementary Files

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