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Anti-inflammatory copper nanoparticles for wound medication

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Abstract: Nanoparticles have received a lot of attention in recent years as a precise and sensitive tool for a variety of applications, including electronic device manufacturing and medical treatments. They played an essential role as a linkage between the atomic or molecular structure and the bulk material, exhibiting new characteristics depending on specific features such as size, shape distribution, capping agent, ionic strength, and morphology. The aim of this work is to investigate the anti-inflammatory and antioxidant properties of biosynthesized copper nanoparticles Cu-NPs in comparison to veterinary drugs as a wound medication in fish skin tissue. A total of 40 fish were collected from Babil Governorate Market and then divided into two groups: 10 as a control group and 30 as an investigating group to make them sick. Later, 30 fish were punched within a syringe and left to get sick for two weeks in a separate water tank. After getting them sick, 10 were treated with Cu-NPs, and another 10 were supplemented with Fungus Guard as a veterinary drug, while the last 10 were left without treatment. The activity of enzymatic antioxidants in fish skin tissue has been used to identify the effect of Cu-NPs in comparison with veterinary drugs as anti-inflammatory agents. After the treatment time, it was discovered that the fish treated with Cu-NPs had significantly improvement in their health than the untreated fish with no signs of infection. The results show that the levels of antioxidant enzyme including SOD, CAT, and GPx increased significantly in infected skin tissue from (0.0241 ± 0.0025, 0.0237 ± 0.0049, and 0.0560 ± 0.0096 U/mg) to (0.1136 ± 0.0049, 0.0638 ± 0.0034, and 0.1174 ± 0.0019 U/mg respectively), after using Cu-NPs as a medication for two weeks with no sign of illness. We can conclude that Cu-NPs act as antioxidants to prevent the oxidative damage due to skin infection.

Key words: copper nanoparticles, inflammation, antioxidant, oxidative stress

1. Introduction

Nanoparticles (NPs) are small particles that range from 1 to 100 nanometres in size, they can exhibit significantly different physical and chemical properties than their larger material counterparts.[1] According to their structural configuration, nanoparticles can be broadly divided into five main categories, like metallic nanoparticles.[2] In general, metal-NPs are solid colloidal metal particles of size range 10 – 1000 nm that incorporate a therapeutic molecule, due to their small size, high surface area to volume ratio, and high heat

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transfer, metallic nanoparticles have been employed and applied in optical, thermal, magnetic, sensory devices, and catalysis. Unique properties of metallic nanoparticles, which offer important economic benefits.[3-5] Copper nanoparticles Cu-NPs, like many other forms of nanoparticles, can be prepared by natural processes or through chemical synthesis, and they have many possible uses in industrial and biomedical fields.[6] Additionally, nano-copper has most important components in medical science because of its numerous therapeutic effects as anti-inflammatory, anticancer, analgesic, and antibacterial properties.[7] Copper (Cu) is a necessary component of all living organisms and is involved in a variety of physiological processes such as protein metabolism, antioxidant activity, cell wall metabolism, and hormone sensing.[8]

It was found that Cu-NPs have remarkable effect on together Gram-negative and Gram-positive bacteria higher than other reported and prepared nanoparticles due to the higher surface-to-volume ratio and relatively small size, which allows interaction closely with microbial membranes.[9] Disease outbreaks in aquaculture as an important limiting factor in production and trade. Chemotherapeutics are drugs which are capable of affecting or killing microorganisms, especially bacteria in the fish culture.[10]

Nowadays there is a great attention for nanotechnology in aquaculture production. It has an efficient role in nutrients and drugs delivery, ponds sterilization, water treatment and aquatic diseases reduction.[11]

Moreover, they have been effectively used in wound dressings, due to their specific physicochemical, optical, and biological properties.[12]

Considering the increased prevalence of multi-drug resistant (MDR) bacteria, NPs with intrinsic potential antibacterial properties, such as Ag, Cu, Au, ZnO NPs, etc., have emerged as potential alternatives for the treatment of bacterial infections.[13] These nanoparticles have complex antimicrobial mechanisms, thus greatly reducing the probability of bacteria developing drug resistance.[14] Furthermore, metallic-NPs can be incorporated into a wide range of wound dressing systems to promote safer and more efficient therapy of infected wounds.[15]

Additionally, some nanoparticles act as antioxidants to remove the action of oxidant, metallic nanoparticles, in particular, have been found to have enzyme-like antioxidant properties which can scavenge free radicals and lower ROS concentrations, which can lead to induce the suppression of pro-inflammatory cytokines, inhibit key signaling pathways and enzymes involved in immune processes.[16]

However, the effect of nanoparticles as a bacterial infection medication and antioxidant actor has been investigated.[13] The present paper presents the role of copper nanoparticles as anti-inflammatory agents in comparison with veterinary drugs and their ability to enhance antioxidant activities of catalase, super oxide dismutase and glutathione peroxidase, which are the main enzymatic protectors against infections.

2. Experimental

2.1. Materials

Tris-HCL buffer, H_2O_2, NaN_3, Tris-EDTA buffer, pyrogallol, potassium dichromate, acetic acid, potassium phosphate, hydrogen peroxide, GSH, HPO_4, DTNB, Na_2HPO_4 and $NaNO_3$. All chemicals and reagents were purchased from Sigma-Aldrich, while, veterinary drugs were purchased from Babil Governorate veterinary clinics.

2.2. Instrumentation

UV-visible spectrophotometer 80 UV-visible spectrophotometer (Bioteh engineering management CO. LTD, UK), Centerifuge Laboratory Retirezle (Centerifuge Laboratory Retirezle) have been used to recognize and analysis data. EDX Laboratory-University of Kashan, Brand:PANalytical, Model:Xpert Pro, Max kv/mA:60/60 has been used to confirm the formation of Cu-NPs.

2.3. Samples collection and preparation

Binni fish from the Babil province market were purchased in February. They weighed 500 grams and were 15 centimeters in length. These fish were then put into fish tanks that are specifically designed for fish farming in the laboratories of the Chemistry

Department. The tanks have dimensions of 70 centimeters long, 60 centimeters wide, and 50 centimeters high. Fish were collected from fish lakes located in Babil province. A piece of tissue was removed at the laboratory site, and homogenized by means of the glass homogenizer submerged in an icy water bath in (100 mM Tris-HCl buffer, pH 7.1) at 4 °C. Samples were centrifuged at 3000 g for 15 min. The supernatant was collected and maintained at −20 °C until the completion of the study.[17]

2.4. Green synthesis of copper nanoparticles

Copper nanoparticles have been synthesized and fully characterized in previous work.[18] Briefly, 60 ml of Artemisia plant extract was added to 200 ml of 0.005 mM copper sulphate solution with constant stirring until the color changed to a flat yellow. The supernatant was purified using a Millipore filter (0.5 M).

Finally, nanoparticle pallets were collected and dried at 80 °C to create a brown precipitate, which was then stored at 4 °C for the next day's work. These nanoparticles had spherical shaped with an average size of 38.5 nm.

2.5. Dosage and sample preperartion

10 infected fish were treated with a 0.05 μg/L veterinary drug while, another 10 were supplemented with the same concentration of Cu-NPs in two different water tanks for two weeks at the same temperature with a capacity of 20 liters each. After two weeks of treatment, a piece of each treated fish tissue was removed from the laboratory site. All samples were homogenized by means of a glass homogenizer submerged in an icy water bath in an ice-cold buffer 100 mM Tris-HCl, pH 7.1. At 3000 rpm for 15 min at 4 °C, homogenized samples were centrifuged. The supernatant was collected and maintained at −20 °C until completion of the study.

2.6. Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined according to the Markland method.[19] A simple and rapid method for the assay of superoxide dismutase is described, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. A plausible explanation is given for the non-competitive part of the inhibition of catechol O-methyltransferase brought about by pyrogallol.

2.7. Determination of catalase activity

The activity was based on the approach of Beer and Sizer method.[20] Catalase catalyses the breakdown of hydrogen peroxide. In the ultraviolet, H_2O_2 absorbs more and more as the wavelength decreases. The decrease in extinction at 240 nm is precisely proportional to the breakdown of H_2O_2.

2.8. Determination of glutathione peroxidase activity (GPx)

The activity of (GPx) was based on the described method by Hafemann *et al.* (1974).[21] The activity of (GPx) was assessed according to an incubation sample with H_2O_2 and NaN_3, Glutathione peroxidase (GPx) oxidizes GSH to produce GSSG as part of the reaction.

3. Results and Discussion

However, the antimicrobial agents from metal nanoparticles against fish pathogens are poorly understood. Hence, the present study has made an attempt to find out the antimicrobial properties of Cu-NPs. After the treatment period, it was found that the treated fish with Cu-NPs displayed a remarkable enhancement in skin health compared to untreated fish. They exhibited reduced symptoms of illness and appeared highest activity as wound medication.

This might be due to the reactive oxygen species (ROS) mechanism. This mechanism can produce significant oxidative stress and alter the cell wall system to equally permeable levels.[22]

On other hand, the fish supplemented with veterinary drugs also showed some improvement in their skin health, but not as significant as those treated with Cu-NPs. They still exhibited some signs of infection.

In order to identify the beneficial role of Cu-NPs, the activity of some antioxidant enzymes was evaluated before and after treatment using two different types

Table 1. The level of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in both healthy and infected fish tissue

Enzyme	Group	N.	Mean ± SD	95 % Confidence Interval for Mean Lower Bound - Upper Bound	Compared groups	P value
CAT (U/mg)	Healthy Fish Tissue	10	0.0972 ± 0.0022	0.0957 − 0.0988	1 and 2	< 0.00001
	Infected Fish Tissue	10	0.0237 ± 0.0049	0.0201 − 0.0272		
SOD (U/mg)	Healthy Fish Tissue	10	0.1473 ± 0.0023	0.1456 − 0.149	1 and 2	< 0.00001
	Infected Fish Tissue	10	0.0241 ± 0.0025	0.0223 − 0.025		
GP_X (U/mg)	Healthy Fish Tissue	10	0.1384 ± 0.0044	0.1352 − 0.1415	1 and 2	< 0.00001
	Infected Fish Tissue	10	0.0550 ± 0.0104	0.0475 − 0.0625		

Table 2. The effect of type treatment on the level of antioxidant enzyme (CAT, SOD and GPx) in infected fish compared to healthy fish

Enzyme	Group	N.	Mean ± SD	95 % Confidence Interval for Mean Lower Bound - Upper Bound	Compared groups	P value
CAT (U/mg)	Healthy Fish Tissue	10	0.0972 ± 0.0022	0.0957 − 0.0988	1 and 2	< 0.0000001*
	Cu NPs Treatment	10	0.0638 ± 0.0033	0.0663 − 0.0614	1 and 3	
	Veterinary Treatment	10	0.0340 ± 0.0073	0.0393 − 0.0287		< 0.00001
SOD (U/mg)	Healthy Fish Tissue	10	0.0972 ± 0.0022	0.0957 − 0.0988	1 and 2	< 0.0000001*
	Cu NPs Treatment	10	0.0638 ± 0.0033	0.0663 − 0.0614	1 and 3	
	Veterinary Treatment	10	0.0340 ± 0.0073	0.0393 − 0.0287		< 0.00001
GP_X (U/mg)	Healthy Fish Tissue	10	0.1384 ± 0.0044	0.1352 − 0.1415	1 and 2	< 0.0000001*
	Cu NPs Treatment	10	0.1067 ± 0.0334	0.1306 − 0.0828	1 and 3	
	Veterinary Treatment	10	0.0807 ± 0.0034	0.0832 − 0.0782		< 0.00001

of fish: healthy and infected fish once, treated and untreated fish subsequently. At the beginning, the level of antioxidant enzymes in each wounded fish was evaluated. The results displayed that infected fish tissue had lower levels of antioxidant enzymes (CAT, SOD, and GPx) than healthy fish tissue. The data indicated a significant reduction in the level of each enzyme, all P-values were < 0.00001 (*Table* 1).

Reducing the activity of each enzymatic antioxidant by about 75 – 80 % in infected fish tissue may be due to the non-conversion of hydrogen peroxide into water and oxygen or the disability of the antioxidant system to handle the excess of harmful oxygen, thus causing inflammatory damage, which happens when cuts or scrapes on the skin get infected by bacteria.[23,24]

Later on, 10 infected fish were treated with a 0.05 μg/L veterinary drug while, another 10 were supplemented with the same concentration of Cu-NPs in two different water tanks for two weeks at the same temperature. A control was handled identically but without exposure to any types of treatment. Fish were fed once every day, and there was no fish death. Finally, the levels of CAT, SOD, and GPx have been determined. The result indicated that the activity of each parameter has been increased by (66, 77, 77 %) respectively, when Cu-NPs are used as a medication with completely healing skin in comparison with veterinary treatment, which was increased by (35, 55, and 58 %), respectively, with incomplete healing. As shown in *Table* 2 and *Fig.* 1.

Increasing the level of catalase, total superoxide dismutase and glutathione peroxidase remarkably after using Cu-NPs compared to veterinary treatment at the same dosage might be due to the size, surface morphology, particle morphology and structure of the nanoparticles. Resulting in, maximum exhibition of Cu-NPs compared to Vet drug at the same treated concentration *Fig.* 2. This action may help to show the ability of Cu-NPs to touch the skin and improve skin's health by making and keeping skin proteins

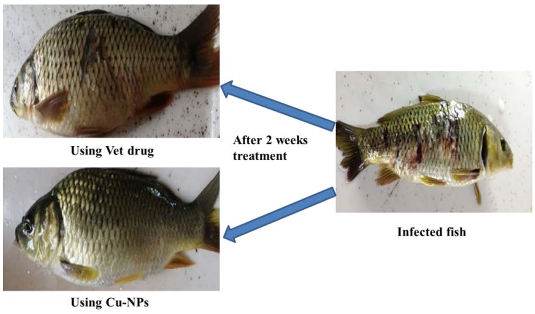

Fig. 1. Skin recovery after treatment with both Vet drug and Cu-NPs.

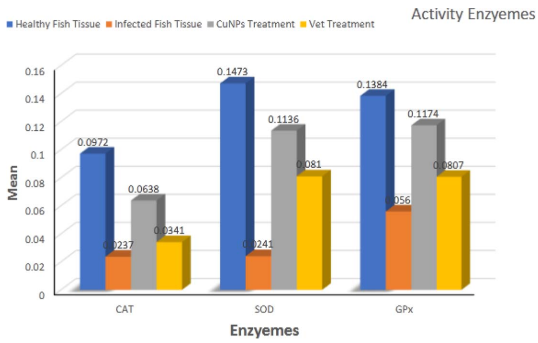

Fig. 2. The activity of CAT, SOD and GPx enzymes in infected fish tissue before and after treatment in compressing with healthy once.

which are necessary for wound healing.[25]

This is because Cu-NPs have the ability to decrease oxidative stress levels, which helps restore the balance between the oxidant and antioxidant systems.[26]

Additionally, the oxidative stress increases the production of lactate dehydrogenase, which is an indicator of cell membrane damage.[27]

4. Conclusions

This work summarizes the progress of metal-NPs-based wound dressings for the detection and therapy of bacterial infections in wounds by maintaining the level of antioxidants, which we believe are the main cause of inflammatory disease.

In this study, the results strongly proved the role of Cu-nanoparticles and showed the possibility of using them as an alternative antibacterial agent for fish skin diseases.

Conflict of Interest

We declare that we have no conflict of interest.

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Ethical Statement

The experiments, which included fish tissues, were approved by the Ethics Committee of the University of Babylon-College of Science-Chemistry department.

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