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Effect of copper sulfate on liver damage induced by nano- zinc oxide in *Cyprinus carpio*

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Abstract

The effects of Copper Sulfate ($CuSO_4$) on repaired the histopthological changes on liver of *Cyprinus carpio* exposed to nano zinc oxide (N-ZnO) was determined in this study.Fish weight 150±10 g divided in to four groups.Fish in first group kept in dechlorinated water while the fish in second group exposed to copper sulfate $CuSO_4$ for 1 hour and in third group the fish was exposed to sub lethal concentration of N-ZnO (30ppm) for 7 days while the fish in fourth group exposed to sub lethal concentration of N-ZnO (30ppm) with CuSO4 (0.3mg\L) for 1hour and then exposed continuously to N-ZnO for 7days. The microscopic examination for the liver done through 24 hour and 7 days from the experiment, which revealed vacuolar degeneration in hepatic cells in fish exposed to N-ZnO for 24 hour also there were dilatation of sinusoid and infiltration of inflammatory cells in liver tissue of fish exposed to to N-ZnO with $CuSO_4$ for 1 hour, there were slight hemorrhage in hepatic tissue and enlargement of hepatic cell in both groups exposed to N-ZnO and $CuSO_4$ for 1 and 24 hour, while the microscopically examination of liver in fish exposed to N-ZnO for 7 days revealed sever pathological lesions characterized by hyperatrophy and necrosis in pancreatic tissue. This study concluded that N-ZnO can cause oxidative stress and usage of $CuSO_4$ at 0.3 mg/L can decrease the toxic effects of N-ZnO liver tissue.

Keywords: Copper sulfate; Liver damage; Nano; Zinc oxide; *Cyprinus carpio*
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تأثير كبريتات النحاس على اذى الكبد المحدث باوكسيد الخارصين النانوي في اسماك الكارب الشائع

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الخلاصة

في هذه الدراسة تم تحديد تأثير كبريتات النحاس في اصلاح اذى النسيج الكبدي في اسماك الكارب الشائع *Cyprinus carpio* والمعاملة باوكسيد الخارصين النانوي N-ZnO. تراوحت اوزان الاسماك بين ١٥٠±١٠ غم والتي قسمت الى اربع مجاميع وضعت اسماك المجموعة الاولى في ماء خال من الكلور في حين تم معاملة اسماك المجموعة الثانية بكبريتات النحاس $CuSO_4$ وبتركيز ٠,٣ ملغم/لتر لمدة ١ ساعة، واسماك المجموعة الثالثة فقد تم معاملتها بالتركيز دون المميت الوسطي لاوكسيد الخارصين النانوي ٣٠ جزء بالمليون ولمدة ٧ ايام، واسماك المجموعة الرابعة فقد تم معاملتها بالتركيز دون المميت الوسطي لاوكسيد الخارصين النانوي ٣٠ جزء بالمليون مع كبريتات النحاس وبتركيز ٠,٣ ملغم/لتر ولمدة ١ ساعة ثم عرضت الاسماك وبصورة مستمرة لاوكسيد الخارصين النانوي ولمدة ٧ايام. وقد اجري الفحص المجهري لنسيج الكبد خلال ٢٤ ساعة و٧ ايام من التجربة والذي اظهر وجود التنكس الفجوي في خلايا كبد الاسماك المعاملة بالتركيز دون المميت الوسطي لاوكسيد الخارصين النانوي ولمدة ٢٤ ساعة وايضا لوحظ توسع الجيبانيات وارتشاح الخلايا الالتهابية في كبد الاسماك المعاملة باوكسيد الخارصين النانوي مع كبريتات النحاس ولمدة ١ساعة ،ووجود نزف طفيف في نسيج الكبدوكبر حجم الخلايا الكبدية في كلا المجموعتين المعاملتين باوكسيد الخارصين النانوي وكبريتات النحاس ،بينما كانت الافات المرضية اكثر شدة قي نسيج كبد الاسماك المعاملة باوكسيد الخارصين النانوي ولمدة ٧ ايام اذ تميزت بالضخامة ونخر النسيج البنكرياسي. وقد استنتج من هذه الدراسة بان

اوكسيد الخارصين النانوي محدث للاجهاد التاكسدي وان استخدام كبريتات النحاس وبتركيز 0.3ملغم/لتر قد ادى الى التقليل من الاثر السمي للاوكسيد الخارصين النانوي.

Introduction

Copper is trace element essential for many physiology development and growth (1), it play an important roles in hemoglobin synthesis,connective tissue synthesis and enzymes involved in cellular respiration as well as in to some structural of protein (2). Copper sulfate has been considered one of the most effective chemical agent used in freshwater aquaculture to control parasitic infection and bacterial disease as well as fungicide and herbicide (3,4).

Copper occur naturally with the environmental at low concentration reached to 0.2-30 µg/L (5), when present in high concentration it become toxic to living organism and fish (6,7). Fish species show beheavioral and locomotor changes and different mortality rate in responses to varing concentration of copper sulfate (8,9) observed that $CuSO_4$ cause damage to the gill epethlium, liver tissue, kidney and spleen of fish, osmoregulation disturbances, immunosuppression and alter blood parameter (9-11).

Nano-scale as nano zinc oxide (N-ZnO) is widely used in sunscreen,so it direct or indirect release to aquatic environmental (12,13) N-ZnO was classify as extremely toxic (14)), the toxicity of N-ZnO depended on species,physicochemical properties of material (15).

Liver and gill might be the target tissue for N-ZnO toxicity (16,17) obsereved that N-ZnO at concentration 30 ppm cause necrosis in both liver and pancreatic tissue in Cyprinus carpio, (18) also reported that N-ZnO cause oxidative stress (OS) in gill, liver, brain, intestine of Cyprinus carpio and cause changes in antioxidant defense mechanism,also there were histopathological changes in gill of Cyprinus carpio treated with different concentration of N-ZnO for 21 days include hyperplasia and degeneration of epithelial cell, vacuolization (19), N-ZnO also toxic for embryos and may cause pericardial edema in zebrafish (Danio rerio) embryos and decrease hatchery rate (20).

N-ZnO have strong absorption ability for organic compound and heavy metals,so the aim of this study was to searching about the effects of $CuSO_4$ on N-Zn0 toxicity on fish *Cyprinus carpio*

Materials and methods

The fresh water fish common carp (*Cyprinus carpio*) weighted 150±10 g were obtained from Agriculture Collage, University of Mosul. They were acclimated to laboratory conditions for 10 days prior the experiments which were done in fish laboratory/Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, the fish kept in dechloraneted water in aquarium 80×80×40cm with photoperiod of a 12:12h and dark cycle,the fish were starved for 24 hr before experiment starting.

Expermental design

The fish (n= 40) were divided in to 4 groups; 1st group of fish exposed to dechloraneted water served as control group, 2nd group of fish exposed to $CuSO_4$, India (dissolved in de-ionized water) at 0.3mg\Lfor 1 hour (21) and then kept in tap water for 7 days, 3rd group of fish exposed to N-ZnO at concentration 30ppm (17) for 7 day after dissolved it in de-ionized water and dispersed with bath for 20 min instead stabilizing agent (22), N-ZnO purchased from the Shijiazhuang Sunpower Technology CO., 4th group of fish exposed to N-ZnO together with $CuSO_4$ (30ppm,0.3mg\L) respectively for 1hour, after that fish exposed only to N-ZnO at concentration 30 ppm for 7 days.

Prepartion of tissue sample

Sample of livers from each group were collected at 1,24 h and 7 day, which were fixed in 10% formaline, routinely processed and embedded in parafine and staining with H&E for light microscop examination (23).

Results

No histopathological changes were observed in the livers of control fish, while the microscopic examination of the liver of fish in groups 2nd and 3rd at 1 hour revealed slight hemorrhage in hepatic tissue and swelling of hepatic cells fig. (1), also there was hepatic cell vaculation in fish exposed to N-ZnO at 24 hour fig. (2). Microscopic examination of liver of fish exposed to N-ZnO with $CuSO_4$ for 1 hour revealed hemorrhage and dilatation of sinusoid and infiltration of inflammatory cells Fig. (3) compared with microscopic changes of liver in fish exposed continuously to N-ZnO only after 24 hour from exposure to N-ZnO with $CuSO_4$revealed only mild hemorrhage and vaculation of hepatic cells fig. (4). These lesions become more sever on fish which are exposed continuously to N-ZnO for 7 day fig. (5) which revealed also hyper atrophy, edema and enlargement of sinusoid with infiltration of inflammatory cells and piknotic hepatic cells with necrosis in pancreatic tissue,

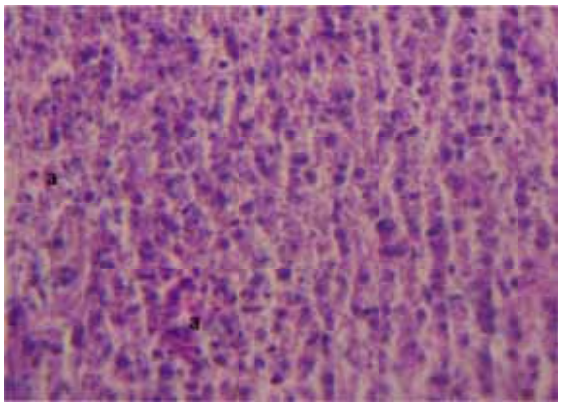

Fig. 1: liver of fish exposed to $CuSO_4$ 0.3mg/Lfor 1hour,revealed slight hemorrhage(a) H&E 420X.

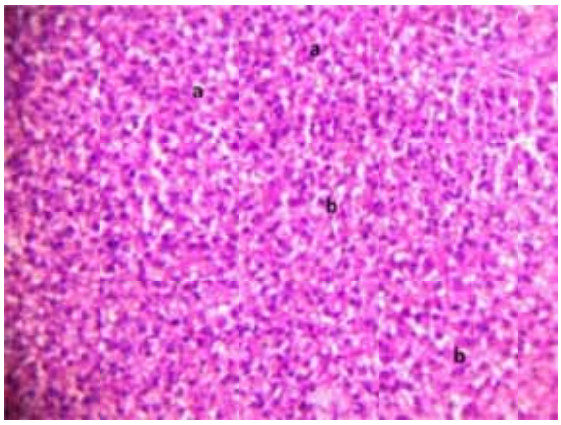

Fig. 2: liver of fish exposed N-ZnO30ppm for 24hour,revealed slight hemorrhage (a) and hepatic cell vaculation (b) H&E 420X.

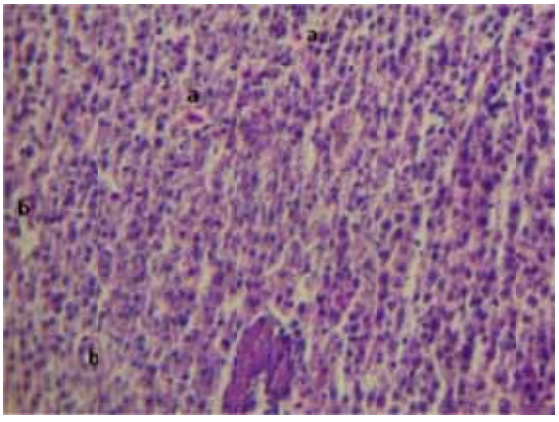

Fig. 3: liver of fish exposed to N-ZnO with $CuSO_4$ for 1 hour revealed hemorrhage (a) and dilatation and congested of sinusoid (b) H&E 420X.

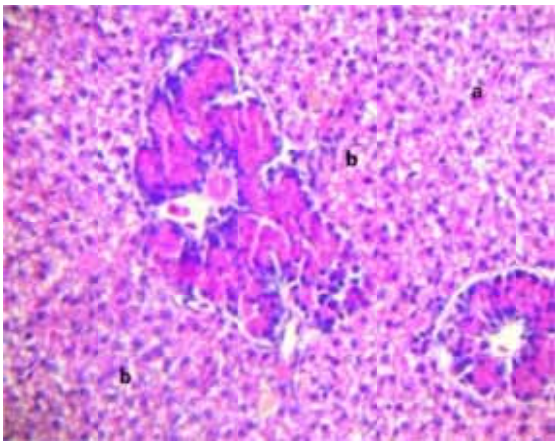

Fig. 4: Liver of fish exposed continuously only to N-ZnO30ppm after 24 hour from exposure to N-ZnO with $CuSO_4$revealed only mild hemorrhage (a) and vaculation (b)H&E 420X.

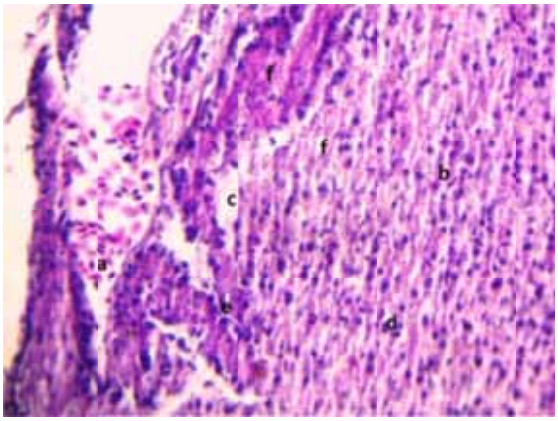

Fig. 5: liver of fish exposed only to N-ZnO30ppm for 7 after exposure to N-ZnO with $CuSO_4$ for 1hour,revealed moderate hemorrhage (a) and dilatation of sinusoid (b) and edema(c), infiltration of inflammatory cells (d), piknosis of hepatic cells (e) with necrosis of liver tissue (f) H&E 420X.

Discussion

Copper was used in the aquaculture industry as additive for control ecto –parasite and algal growth (24), $CuSO_4$ is highly toxic to fish even at recommended rate of application (25), the toxicity and bioavailability of Cu depended on water parameter as PH, alkalinity and concentration of dissolved particulate organic matter (26,27). The histopathological results of all fish treated with N-ZnO and $CuSO_4$together, also in fish exposure to $CuSO_4$ are similar to those result of (25) who observed that the $CuSO_4$ could cause oxidative stress and alteration in an

antioxidant defense mechanism in gold fish Carassius auratus after 24 hrs exposure to $CuSO_4$. As dissolved Cu^{+2} was eliminated from pond water after a short period and the rate of eliminated was variable according to water qualityand it is electro statically attracted to organic or inorganic molecules form stable complexes that are more toxic to fish (28).

Nanoparticles are disseminated to all body organs (29) and the liver is more important organ which is play a critical role in metabolism, excretion and detoxification (30). So in this study there were negative effects of N-ZnO in liver architecture in all treatment but these lesions were more severity in fish which was exposure to N-ZnO for 7 day,these result were agreement with study of (17) who observed the effects of lethal concentration N-ZnO in common carp there were liver necrosis and hemorrhage with degeneration in pancrease tissue,also (31) observed nercosis in liver and gill of Oreochromius mossambicus. The result of (32) showed that the toxicity of N-ZnO could occur after a short period of exposure which accumulated in liver and the degree of damage had the time dependent, so our results were more sever in fish exposure to N-ZnO for 7 day than other groups. The size of nanoparticles was considered the most important key factors influencing the toxic effect of nanoparticles (33) and have the ability to activation granulocyte and thrombocyte and induced inflammation and hemolysis (34).The histopathological alteration which were revealed in our study may be duo to the ability of N-ZnO to induced the cellular oxidative stress(OS) which was the main toxic mechanism of N-ZnO (35) these (OS) was interact with oxidative organells as mitochondria (36) and other biomolecules and cause imbalance between the production of reactive oxygen and the biological systems ability to detoxify reactive intermediates or repair, these disturbances lead to tissue damage (37). Study concluded that N-ZnO can cause oxidative stress and usage of $CuSO_4$ at 0.3 mg/L can decrease the toxic effects of N-ZnO liver tissue.

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