PATHOLOGICAL STUDY OF LETHAL CONCENTRATION OF N-ZNO IN COMMON CARP *CYPRINUS CARPIO* L.

S.K.I.AL-Taee A.H.A.AL-Hamdani

Department of pathology , College of Veterinary, University of Mosul , Mosul, Iraq

(Received 24 March 2013, Accepted 15 May 2013)

Keywords; mortality, necrosis, N-ZnO.

ABSTRACT

The lethal concentration of N-ZnO was determined in this study ,juvenile *Cyprinus carpio* L.mean weight 150±10g which were exposed to different concentration of N-ZnO (10-50 PPM) for 24 hrs ,the mortality rate of fish reached to 100% at 50 ppm concentration while the percentage decreased to 50% when the fish exposure to 30ppm concentration of N-ZnO.The histopathological lesions in the kidney were characterized by hemorrhage in an interstitial nephrotic tissue ,infiltration of inflammatory cells , there was cloudy swelling in the renal tubules . In the gill there were hyper trophy of pillar cells and necrosis in the secondary gill filaments .There were necrosis in the hepatic tissue and hemorrhage and sloughing in the pancreatic tissue.

INTRODUCTION

Nanoparticle represented an intermediate supramolecular state of matter between bulk and molecular material their diameter 100 and 1 nm(1) . Nanoparticls(NP) have physicochemicle properties as large surface area and their small size make them more toxic than their bulk because of their ability to penetrate cells and accumulate in it (2 and 3).Nanoparticle released to the environment through washing consumer products ,spilling or drug synthesis and drug delivery(4). Zinc Oxide nanoparticles(N-ZnO) was one of the most commonly used types of metal –based, It has been demonstrated that the N-ZnO were classified as extremely toxic (5) it was toxic to aquatic environment (6),it's toxicity may be due to it is greater specific area (7) ,dissolved zinc ion (6)and surface charges were found to be important in initiating contact between

the nano material and cells (8) , these may cause mechanical oxidative stress(9)or genotoxicity (10). Small size of N-Zn easy entry to fish through gill or ingestion and cause tissue damaging (11). N-ZnO affected fish at early stage (12) observed that the N-ZnO (0.1-100mg/L) for 96hpf cause decreased in hatching rates with pericardial edema ,nanometals in water column could cause respiratory toxicity ,mucous secration and internal organ pathology(13).

The aim of this study has been determined the lethal concentration of N-Zn-O on *Cyprinus carpio* and to study it effects on the some tissue.

MTERAILS AND METHOD

1-Fish

Juvenile *Cyprinus carpio* with mean length 70±5cm and mean weight 150±10g were obtained from collage of Agriculture and forestry /University of Mosul, fish were acclimated in dechlorinated tap water for at least 7 day in the laboratory before experimental time and fed twice daily ,the water temperature was mentained at 25 ±2c°.

2-Preparation and characterization of N-ZnO.

N-ZnO was purchased from the Shijiazhuang Sunpower Technology CO., it's purity and characteristics were listed in the table (1)

Table (1) Shows characterization of N-ZnO

Specification	Content Zn O%	Metallic Zinc	Pb%	Mn%	Cu%	AS%	Cd%
95%	95.2		0.03	0.005	0.003		
	Hg%	Water soluble matter%	HCl insoluble matter%	Speciefic surface area	Particle Size/nm	105C° volatile matter %	Ignition Loss%
		0.7	0.05	3.6	50	0.7	4.0

Suspantion of the N-ZnO was prepared with aerated single –distilled water and dispersed with bath for 20 min instead stabilizing agent(14)

3-Lethal toxicity studies

The lethal concentration LC50 of N-ZnO which was determined through using Trevan method (15) the toxicity of N-ZnO was investigated byexposure fish *Cyprinus carpio* to different concentration of N-ZnO (10-50 ppm) for 24 hrs ,10 fish for each concentration have been used .

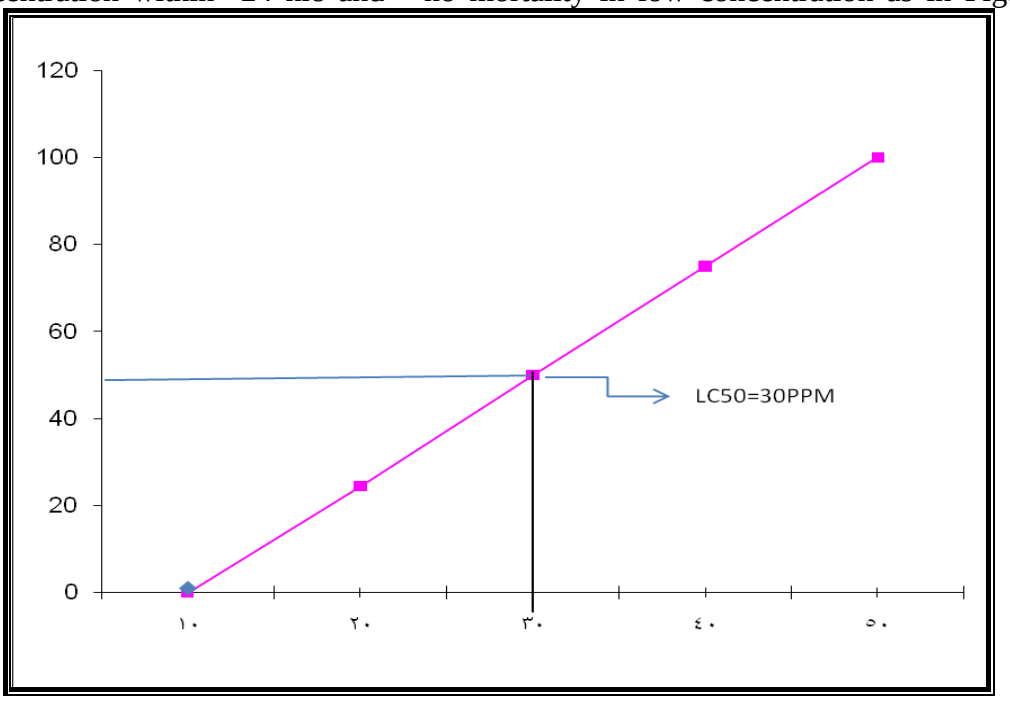
4-prepared samples

Tissue samples from kidney ,gill,liver were taken (16)and fixed in buffer formaline10% for histopathological examination and stained with H&E(17).

RESULTS

Lethal toxicity

The lethal concentration of N-ZnO was 30ppm concentration which led to kill 50% of *Cyprinus carpo* ,while the mortality rate of fish reached to 100% in 50ppm concentration within 24 hrs and no mortality in low concentration as in Fig.(1)



STANDER CURVE FOR NANO ZINC OXIDE "PPM" Fig.(1)

Clinical signs :

The clinical observation of the affected fish were nervous signs ,abnormal swimming ,jumping and gasping .

Gross pathology:

There were white necrotic foci in kidney with g paleness of gill and liver .

Histopathological examination :

Microscopic observation of the kidney in the fish treated with 30 ppm of N-ZnO revealed hemorrhage in an interstitial nephritic tissue ,infiltration of mononuclear cells , there was cloudy swelling in the renal tubules fig.(2).In gill there was hypertrophy of pillar cells and hemorrhage and necrosis in the secondary gill filaments fig.(3), while in liver there were pathological changes characterized by necrosis in the hepatic tissue , hemorrhage and infiltration of mononuclear cells,in pancreas there was hemorrhage and sloughing fig.(4,5)

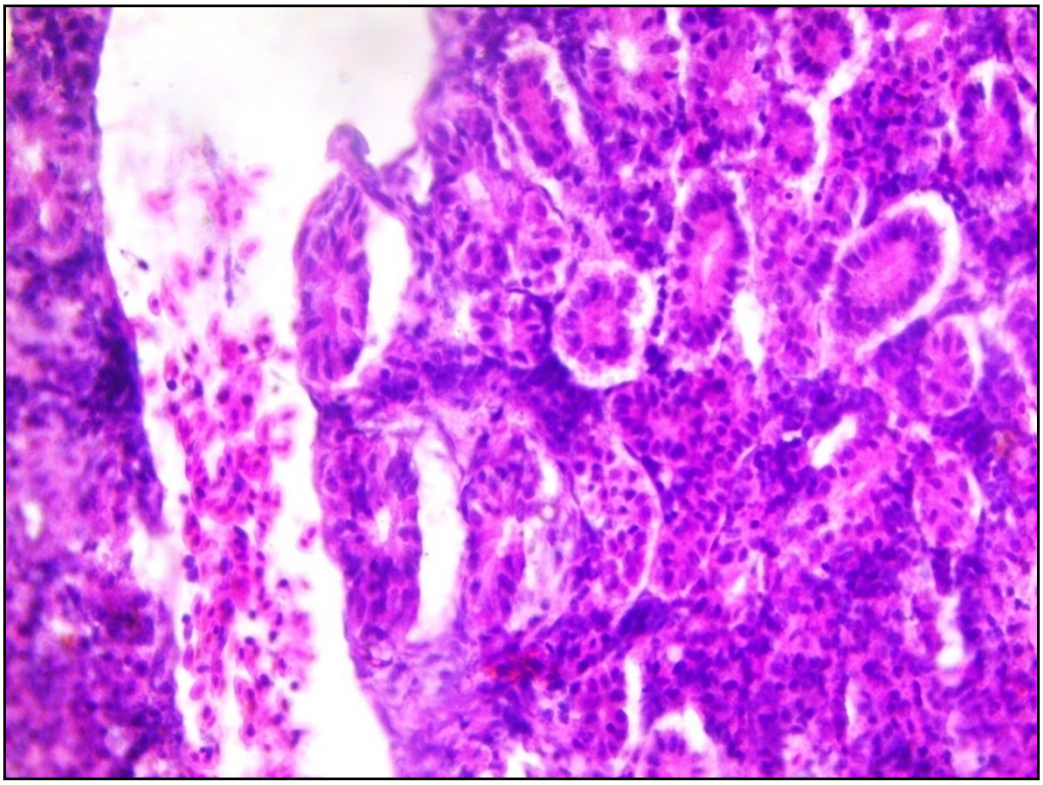


Fig.(1) kidney of fish treated with 30ppm of N-ZnO showing cloudy swelling(a) hemorrhage (b) ,mononuclear cells infiltration(c) and edema (e),H&E.105X

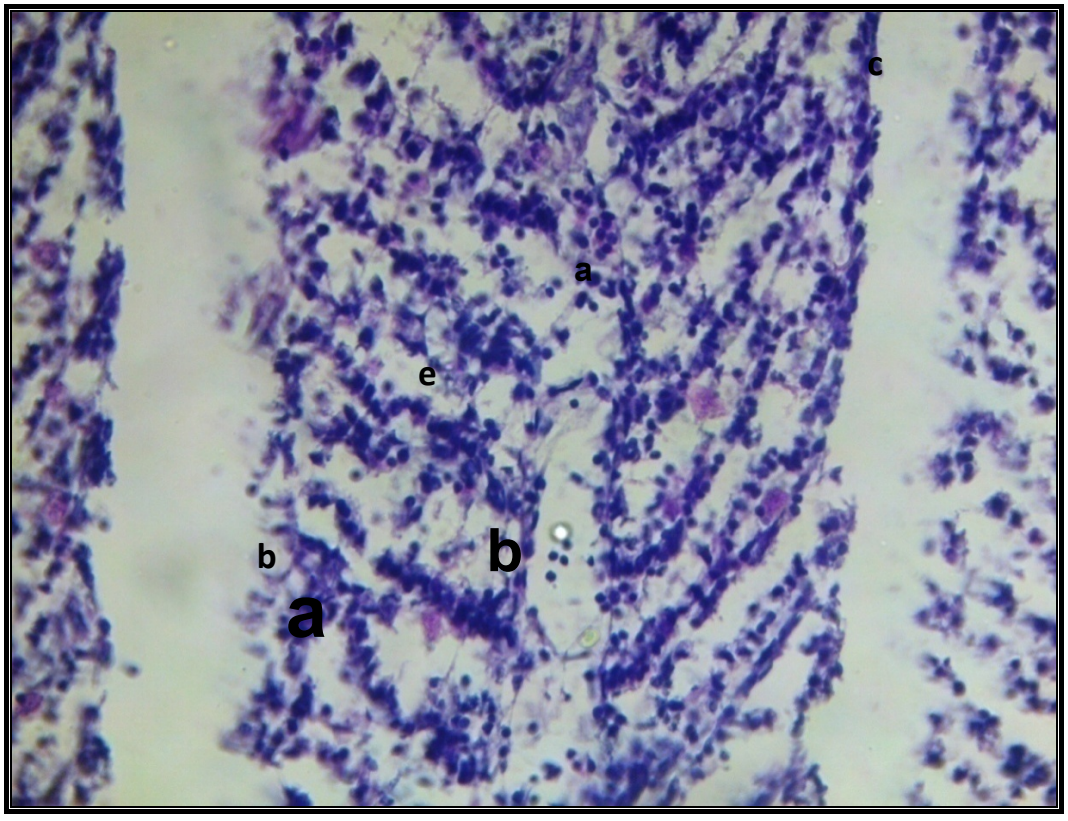


Fig.(2)gill of fish treated with 30ppm of N-ZnO showing hypertrophy of secondary gill filaments(a) and necrosis (b) ,H&E.105X

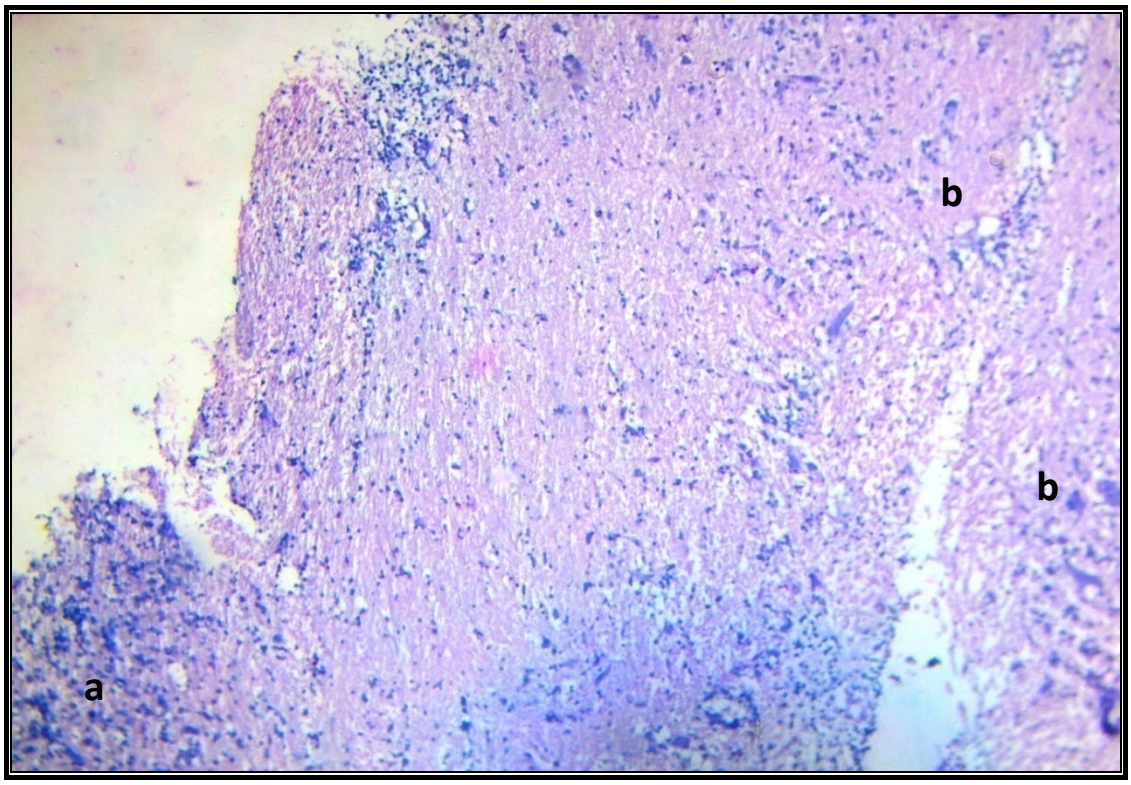


Fig.(3)liver of fish treated with 30ppm of N-ZnO showing inflammatory cell infiltration (a) and necrosis (b),H&E.105X

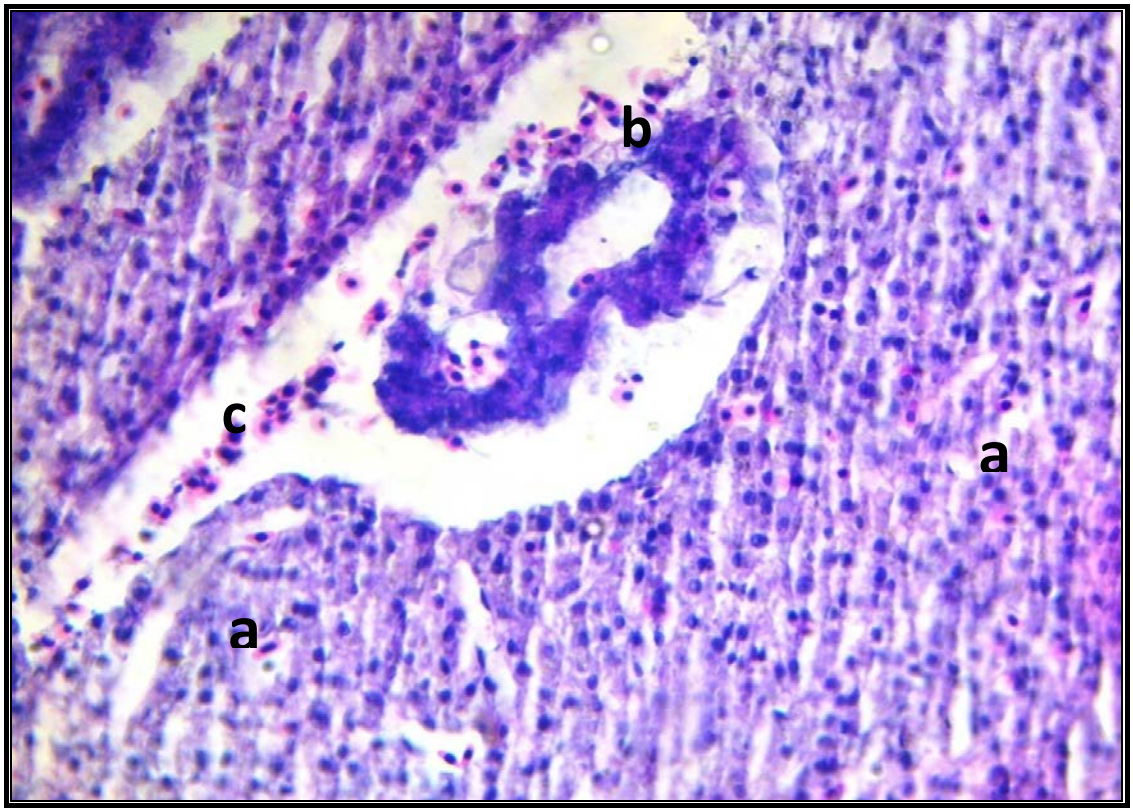


Fig.(4)liver of fish treated with 30ppm of N-ZnO showing necrosis (a) sloughing of pancreatic tissue (b) and hemorrhage(c) H&E.1440X

DISCUSSION

Nano particle are represented intermediated state between bulk and molecular material, they exhibit toxicity more than bulk because their greater surface reactivity and small size have ability to penetrate and accumulate with in cells (3,18) .Another crucial factors that play important role on N-ZnO toxicity were their ability to cause damaging for cell membrane through reactive oxygen species ROS mechanism (19) and release metal ion which is the main cause of cell swelling (6, 20).

The pathological lesion which have been observed in our study may be due to the ability of N-ZnO to cause increase cellular oxidative stress response and lead to changes in the activities of antioxidant enzyme as superoxide dismutase SOD ,catalyses enzyme CAT and glutathione also there was reduction in the glutathione level and increase in lipidperoxide LPO gill,liver and brain of juvenile *Cyprinus carpio* when exposed to different concentration of N-ZnO (21,22).

Other previous study has been shown that N-ZnO cause necrosis of different organs of tissue of *Oreochrois mossamibcus*,this result could indicate that the N-ZnO have ability to cause tissue damage and ultimately caused to death (11).

دراسة مرضية للتركيز المميت لصغائر اوكسيد الخارصين في اسماك الكارب الشائع

Common carp (*Cyprinus carpio* L.)

شهباء خليل ابراهيم الطائي ألاء حسين علي الحمداني

فرع الامراض وامراض الدواجن ، كلية الطب البيطري ، جامعة الموصل ، الموصل ، العراق .

الخلاصة

في هذه الدراسة تم تحديد التركيز المميت لصغائر اوكسيد الخارصين في اسماك الكارب الشائع *Cyprinus carpio* L والتي كان معدل وزنها 150±10 غم .اذ عرضت الاسماك الى تراكيز مختلفة من صغائر اوكسيد الخارصين (30–50) جزء بالمليون ولمدة 24 ساعة .وقد كان معدل نفوق الاسماك 100%عند تركيز 50 جزء بالمليون، في حين كان معدل النفوق 50% عند تركيز 30 جزء بالمليون . ولقد لوحظ حدوث تغييرات مرضية نسجية في الكلية والتي تمثلت بوجود النزف في النسيج الخلالي وارتشاح الخلايا الالتهابية وحدوث التورم الغيمي في النبيبات الكلوية.اما في الغلاصم فقد لوحظ تضخم الخلايا نوع Pillar cells ونخر الصفائح الغلصمية الثانويةوفي الكبد فقد لوحظ حدوث النخر مع وجود النزف للنسيج البنكرياسي.

REFERANCE

1-Mair ,T.;Korting,H.(2005).Sunscreen –which for ?Skin Phamacol Appl Skin Physiol.18;253-62.

2- Lovern S.B.;Klaper R.(2006).Daphania magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticle .Environ Toxicol Chem.,25:1132-7

3-Mironava T.,Hadjiargyrou M.,Simon M.,Jurukovski V.,Rafailovich MH.(2010).Gold nanoparticles cellular toxicity and recoveryeffect of size ,concentration and exposure time .Nanotoxicology ;4:120-37.

4-Dhawan A.,Sharma V.(2010).Toxicity assessment of nanomaterials :methods and challenges.Anal Bioanal Chem 398:589-605.

5-Kahru A.,Dubourguier HC.(2009).From ecotoxicology to nanotoxicology. Toxicology ,10;269(2-3):105-19.

6-Aruoja V., Dubourguier H.,Kasemets K.,Kahru A.,(2009).Toxicity of nanoparticlesof CuO,ZnO and TiO_2 to microalgae *Pseudokirchmeriella subcapitata* .Sci Total Environ 407:1461-1468.

7-Adams L.,Lyon D.Y.;Alvares. PJJ.(2006).Comparative ecotoxicity of nanoscales TiO_2 ,SiO and ZnOwater suspensions.Water Res,10;3527-3532.

8-Neal AL.(2008).What can be inferred from bacterium –nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles ?Ecotoxicol 17:362-371.

9-Xia T, Kovochich M, Brant J, Hotze M, Sempf J, Oberley T, Sioutas C, Yeh JI, Wiesner MR, Nel AE (2006) Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett 6:1794–1807

10-Yang H, Liu C, Yang D, Zhang H, Xi Z (2009) Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. J Appl Toxicol 29:69–78

11-Amutha C. and Subramanian.(2009).Tissues damaging effect of Zinc Oxide nanoparticles on *Oreochromis mossambicus* .Biochem Cell Arch. 9:2;235-239.

12-Zhu; Zhu X.;Wang J.;Zhang X. and Chang Y. and Chen.(2009).The impact of ZnO nanoparticle aggregates on the embryonic development of zebrafish (*Danio rerio*) .Nanotochnology,20,195103 Available from http://dx.doi.org.

13-Handy R.D.,AL-Bairuty G.,AL-Jubory A.,Ramsden C.S.,Boyle D.,Shaw B.J. and Henry T.B.(2011).Effects of manufactured nanometerials on fish :a target organ and body systems physiology approach.Journal of Fish Biology ,79(4);821-853.

14-Xiong D.;Fang T.;Yu L.;Sima X. and Zhu W.(2011).Effects of nano-scales TiO_2 and ZnO and their bulk counterarts on zebrafish:Acute toxicity ,oxidative stress and oxidative damage.Science of the Environment 409:1444-1452.

15-Mohammad,F.K.and AL-khafajee ,N.J.(2001).Veterinary toxicology. Mosul university , Mosul, Iraq ,p21

16-Lucky Z.(1977).The diagnosis of bacteria disease by infection experiments.In Hoffman G.L.(ed)methods for diagnosis of fish disease ,Amerin and New Delhi p40.

17-Luna L.G.(1968).Manual of histological staining methods of the Armed Forces institute of pathlogy .3rd ed.The Black stone Division,McGraw-Hill Book Comp .,NewYork.

18-Hoet P.H.M.;Brüske-H.and Salata O.V.(2004).Nanoparticles-known and un knownhealth risks.J.Nanobiotechnology 2,12(doi:10,1186/1477-3155-2-12).

19-Zhang L.,jIANGy.,Ding Y.,Povy M.,York D.,(2007).Investigation in to the antibacterial behaviour of suspension of ZnO NANOPARTICLES(zNo nanofluids) .J.Nanopart Res 9:479-89.

20-Nair S.,Sasidharan A.,Nair S. and Raina S.(2008).Role of size scale of ZnO nanoparticles on toxicity toward bacteria and osteoblast cells .J.Mater sci.20,S235-S241.

21-Hao, L. ; Chen, L.Hao, J. and Zhang, N.(2013).Bioaccumulation and sub-toxicity of zinc oxide nanoparticles in juvenile carp (Cyprinus carpio):A comparative study with its bulk counterparts.Ecotoxicol Environ Saf.pii:S0147-6513(13)00010-9.doi

22- Hao, L. and Chen, L.(2013).Oxidative stress responses in different organs of carp (Cyprinus carpio)with exposure to ZnOnanoparticles Ecotoxicology and Environmental Safety volume 80 ,ISSN0147-6513.