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Project paper on: The effect of zinc exposure on the histology of liver and kidney.

The project paper is submitted to Addis Ababa University, School of Medicine; Department of Anatomy in partial fulfillment for the degree of Masters of Science in Anatomy.

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List of Abbreviations

Al – Aluminum

ALP- alkaline phosphatase

ALT- alanine amino transferas

ANOVA -Analysis of variance

AST- aspartate amino transferase

ATSDR -Agency for Toxic Substances and Disease Registry)

B.W-body weight

BUN- blood urea nitrogen

CR- creatinine

EDTA-ethylene diamine tetra acetic acid

Fig – figure

H&E – Haematoxylin and Eosin

Hb -hemoglobin

Kg- kilogram

LC50- lethal concentration

LDH - lactate dehydrogenase

LSD -least significant difference test

MCH-- mean corpuscular hemoglobin

MCHC- mean cell hemoglobin concentration

MCV- mean corpuscular volume

Mg- milligram

ppm - Parts per million

Zn –zinc

ZnSO4. 7H2O - zinc sulphate (hydrated)

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Summary

Zinc is one of the most important trace elements in the body that participates in the biological function of several proteins and enzymes. Despite the fact that small quantity of zinc is required for the normal development and metabolism, its effect is toxic when a certain concentration is exceeded. The aim of this paper is to review scientific literatures on the protective and toxic effect of zinc exposure on the histology of liver and kidney. The studies were evaluated in relation to dose of zinc, zinc combination with other toxic metals, duration of exposure, type of animal model used, method of study used and parameter used to measure protective effect of zinc against toxic metals and toxic effect of zinc alone. The different literatures reviewed in this paper used rats, mice, lambs, fishes, ducks. The literature reviewed showed that Zn administration together with Al, Cd, as well as organic solvent such as ethanol has a protective effect against Al, Cd induced toxicity in liver and kidney tissues and ethanol induced toxicity in liver tissue. On the other hand, as zinc dose level and duration of the exposure increases, it act as toxic metal to histology of liver and kidney.

Key words: zinc, liver, kidney

1. Introduction

1.1. General over view of zinc

Zinc is a lustrous, blue-white metal occurs naturally in the environment and constituting 20–200 ppm (by weight) of the Earth's crust and is found as zinc oxide, Zinc chloride, Zinc sulfate (ATSDR, 1995). Because of its reactivity, zinc metal is not found as the free element in nature. In the periodic table of the elements, it can be found in group IIb, together with the two toxic metals cadmium and mercury. Nevertheless, zinc is considered to be relatively non-toxic to humans (Fosmire1990).

Table 1: Chemical and physical properties of zinc and zinc compounds

	Zinc	**Zinc oxide**	**Zinc chloride**	**Zinc sulfate**	**Zinc sulfide**
Molecular formula	Zn	ZnO	ZnCl2	ZnSO4	ZnS
Molecular weight	65.38	81.38	136.29	161.44	97.44
Melting point, ^{o}C	419.5	100(decomposes)	283	600 (decomposes)	~1700
Boiling point, ^{o}C	908	No data	732	No data	No data
Water solubility, g/L (25^{o}C)	Insoluble	~$2x10^{-3}$	$4.3x10^{3}$	$1.7x10^{3}$	~$7x10^{-3}$
Density (g/cm3)	7.14	5.607	2.907	3.54	~4.1

Source: ATSDR, (1995)

1.2. Occurrence and source of zinc

Zinc is an essential element in the nutrition of man, animals and plants. It functions as an integral part of numerous enzymes. Because of its essentiality, zinc is present in all plant and animal tissues. The total body zinc for a 70 kg individual has been estimated to be 2.3 g. Protein foods are important dietary sources of zinc since they contain a large amount of enzymes that form complexes with zinc. Zinc is also present in the diet as inorganic salts such as the sulphate, oxide or gluconate. (WHO, 1973)

1.3. How human are exposed to Zinc

There are three major routes of entry for zinc into the human body; by inhalation, through the skin, or by ingestion [ATSDR, 2005]. However, absorption efficiencies for these routes have not been quantified in humans or animals each exposure type affects specific parts of the body and allows the uptake of different amounts of zinc.

1.4. Health effects of Zinc

Zinc is one of the most important trace elements in the body, and participates in the biological function of several proteins and enzymes (Maity *et al*., 2008). Despite being an essential trace element, Zinc is toxic to most organisms above certain concentrations. Although, small quantities of zinc are required for the normal development and metabolism, if its level exceeds the physiological requirements, it can act as a toxicant**.** Zinc deficiency results in failure of keratinization, which leads to parakeratosis, loss and failure of growth of wool and hair, lesions of coronary bands, retarded testicular development and cessation of spermatogenesis in zinc deficient animals (Kendall *et al*., 2000).

1.5. Absorption and elimination of zinc in body

Zinc, as contained in food and drink, is absorbed through the gut mucosa. Absorption of zinc through the gastrointestinal tract is homeostatically controlled. Absorption of zinc salts from food is approximately 20-40%, being higher from flesh food but lower from cereals, where phytate content impairs absorption (WHO, 1982). Zinc is absorbed both by passive diffusion and an unknown membrane carrier process, which requires energy. The initial site of absorption is the stomach where absorption occurs within 15 minutes of ingestion; however, the major site is the second portion of duodenum. Absorption also occurs in other segments of both the small and large intestine (Henkin & Aamodt, 1975).Once absorbed, zinc is distributed throughout the body, with most (90%) found in muscle and bones. Many substances can affect the absorption of zinc. Both phytate and soy protein inhibit the formation of the zinc-protein complex and as a result diminish the absorption of zinc (WHO, 1982). Other substances that adversely affect zinc absorption include cottonseed, peanut, safflower, calcium, phosphate, food and zinc itself (WHO, 1982). Iron and copper compete for absorption with zinc. The absorption of zinc has been shown to be enhanced by the presence of histidine, cysteine, methionine and ethylenediamine tetracetic acid. These are thought to act by promoting the formation of the low molecular weight organic zinc complex (WHO, 1982).

The major route of zinc elimination is via the faeces with minor amounts eliminated in the urine, semen and sweat. Administration of large amounts of zinc does not result in elevated tissue levels, since increasing the level of zinc in the diet results in decreased absorption (WHO, 1982).

1.6. Major uses of zinc

Zinc metal is used most commonly as a protective coating of other metals, such as iron and steel. Zinc is also a component of various alloys including those used for die casting as well as brass and bronze. Zinc salts have numerous applications and are used in wood preservation, catalysts, and corrosion control in drinking water systems, photographic paper, vulcanization acceleration for rubber, ceramics, textiles, fertilizers, pigments, batteries, and as nutritional supplements or medicines (ATSDR, 1995). Zinc chloride is a primary ingredient in smoke bombs used for crowd

dispersal, in fire-fighting exercises (by both military and civilian communities), and by the military for screening purposes. Zinc chloride, zinc sulfate, zinc oxide, and zinc sulfide have dental, medical, and household applications. Zinc chloride and zinc sulfate are also used in herbicides (ATSDR, 1995).in addition zinc is extraordinarily useful in biological systems. It was found in all body organs and is needed for normal hair; skin feather and body bone growth and also plays an important role in the immune system, regulation of appetite, stress level, taste and smell (McClain et al. 1992).

1.7. Potential for Zinc Accumulation

An average 70-kg adult contains 2-3 g of zinc, making it nearly as abundant as iron. The zinc is widely distributed in the skeletal muscle, bone, brain, GI tract, liver, kidney, lung, heart, retina, pancreas, sperm and uterus. The highest concentration (100 mg/kg wet weight) is found in the prostate. Concentration of zinc in whole blood is about 5 mg/L and about 5-fold less in plasma and serum. Unlike iron, there is no particular body store for zinc and metabolic zinc requirement must be met by intake of food and supplements coupled to poorly understand homeostatic processes. (Nriagu J, 2007)

The body controls the amount of zinc stored in the body by reducing the absorption and increasing excretion when intakes are increased above the metabolically set threshold. The distribution of zinc in some tissues may be regulated by age to some degree. Zinc concentrations increase in the pancreas, liver, and prostate but decrease in the aorta and uterus with age. Levels of zinc in the kidney and heart tend to peak at about 40–50 years of age and then decline. (Nriagu J, 2007)

2. Review and critical analysis of published research on the effect of zinc on histology of liver and kidney.

A number of researches using different animal models, and using various methodologies have been conducted on the effect of zinc and zinc compound exposure on histology and function of liver and kidney. Some of these studies have been reviewed and analyzed in this project paper.

A research conducted by Gawish, (2005) investigated the effect of Zinc Sulphate on the toxicity of Aluminum Sulphate in Liver and Kidney of Male Albino Rats.

In this study forty five adult male albino rats weighing 160-170g were used. The animals were divided into, three groups of fifteen rats each. Group one, group two and group three animals served as the control, aluminum sulphate treated and aluminum sulphate plus zinc sulphate treated group respectively.

Group two animals received 50mg/kg/day of Aluminum sulphate daily for 45 days orally. Group three animals were given 50mg/kg/day of Aluminum sulphate and 50mg/kg/day of zinc sulphate. At the end of the treatment period, rats of each group were sacrificed and samples of liver and kidney were collected and fixed in aqueous Bouin solution for histological studies. Six mm thickness paraffin sections were prepared and stained with Ehrlich Haematoxylin & Eosin .With this set up histological analysis was done by using light microscopic examinations.

The results of this investigation showed that liver tissue in Aluminum sulphate treated group showed mild histological changes, some swollen cells and empty area appeared around the nucleus; inflammatory cell and some fragmentation of chromatin materials were appeared. At the end of the experiment (45 day) empty spaces within the cytoplasm and dense bodies within the nuclei were appeared compared to the control group. However Zinc sulphate plus Aluminum sulphate treated group showed some improvement in liver tissue in which hepatocytes appeared almost enlarged blood sinusoids normal compared to Aluminum sulphate treated group.

Kidney tissue of Aluminum sulphate treated group showed various histological changes where slight shrinkage in the corpuscles, congestion of renal corpuscles were appeared, and renal tubules cells loosed their normal shape indicating distortion in their structures at 45 day of administration compared to control group. However Aluminum sulphate plus zinc sulphate treated group showed beginning of amelioration of kidney tissue at 15 day of treatment,

continued with the effect of zinc, at 30 day of treatment less shrank corpuscle was observed; but at the end of experiment (45day) the effect of Aluminum sulphate was still affected the renal tubules and corpuscles in which zinc cannot appear improvement on kidney tissue.

Another investigation by Najafzadeh *et al*, (2013) studied the effect of zinc oxide nano-particles on serum biochemical factors and histological make up of the liver and kidney in lambs. In this study eight male lambs, aged 5 - 6 months and weighing 18-20 kg were used. The animals were divided into two groups of four lambs each. Group one and group two animals served as the control, and experimental groups respectively.

Both groups of lambs were allowed free access to diet and water. After one week of acclimation, the suspension of zinc nanoparticle was orally administrated to lamb of experimental group at a dose of 20mg/kg body weight daily for 25 days. Blood was collected from vein before and in the end of study and serum was separated by centrifuge. These samples were stored at -20 C until analysis. The biochemical levels including lactate dehydrogenase (LDH), alanine aminotransferas (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CR), were assayed. Level of zinc in serum of lambs was determined at first day and 25 days. The animals were sacrificed on 25th day. The liver and kidneys tissues were collected, and fixed in 10% formalin-buffer for histological examination. Then, tissues embedded into paraffin, sectioned for 5 μm thick, and mounted on the glass microscope slides using standard histopathological techniques. The sections were stained with hematoxylin-eosin and examined by light microscopy.

Statistical analysis were carried out by one-way Analysis of Variation (ANOVA) and Fisher least significant difference test (LSD) was tested for significant differences between groups. A significant difference was considered to be $p< 0.05$.

The results of this investigation showed that there was no mortality in zinc nanoparticle group. The amount of serum zinc and the effect of oral administration of zinc nanoparticles on the serum biochemical levels of lambs are shown in Table-2-. When zinc level results were evaluated it was observed that, before and after oral exposure to zinc nanoparticles showed an increase zinc level but this sharp elevation was not significant ($p<0.05$). Table-1shows The mean concentration of Zn in serum at the end of the experiment was 2.02 μmol/L in nano zinc group whereas before nano zinc supplement the mean values were around 0.79 μmol/L .The

results indicated that activity of creatinine was significantly increased compared with the before treatment (P= 0.002). The level of the serum ALP was significantly decreased after administration of zinc nanoparticles (p=0.011). The level of other factors did not change Significantly (Table-2).

Table 2. Effect of zinc nanoparticle on concentration of serum parameters.

	Zn (µmol/L)	ATL (IU/L)	AST (IU/L)	ALP (IU/L)	LDH (IU/L)	Creatinine (mg/dL)	BUN (mg/dL)
Day0	0.79±0.09	18.66±3.71	86.66±14.24	469.5±105.40*	1055.5±209.79	1±0.04*	15±1.87
Day25	2.02±0.78	16±0.57	91±29.67	248±64.16	1008.2±288.95	1.85±0.11	19.5±3.57

Results are presented as Mean ± SE.

* Represents significant difference at $p<0.05$.

The histopathological pictures of liver are illustrated in Figure-1. At the microscopic level, the liver of lambs nano zinc group presented the reversible histopathological changes as cell swelling (about 50% of lambs was seen) and irreversible histopathological changes as eosinophilic necrosis of hepatocytes (about 50% of lambs was seen). Figure-2 shows histopathological findings of kidney control and nano zinc group. Significant histopathological alteration of kidney tissues nano zinc group was multifocal interstitial nephritis (about 75% of lambs was seen), but remainder of kidney nano zinc group was normal. Serum level of zinc was increased by oral administration of nano zinc particles in lambs in their study but this elevation was not significant. Therefore, in this study the significantly did not elevate activity of these enzymes but ALP level was significantly decreased and its reason is not clear but may be related to growth of lambs.

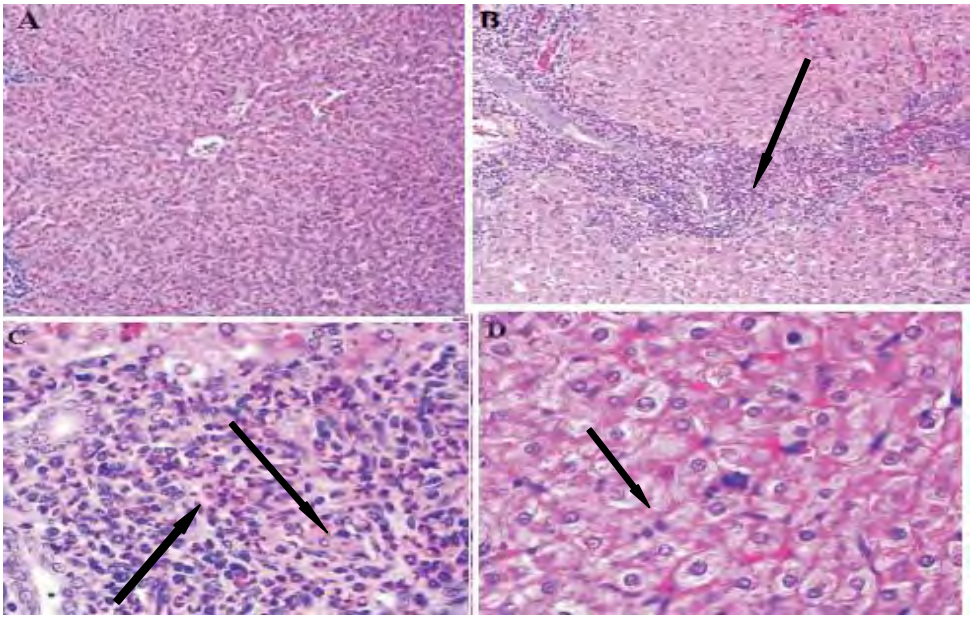

Figure 1. Liver tissues from control group and nano-zinc treated group lambs

(A) Liver in the control group, showing normal structure. (B) Liver in the nano zinc treated group. Arrows show eosinophilic necrosis of hepatocytes. (C) Liver in the nano zinc group. Arrows show eosinophilic cells. (D) Liver in the nano zinc group. Arrow show cell swelling hepatocytes. (H&E).

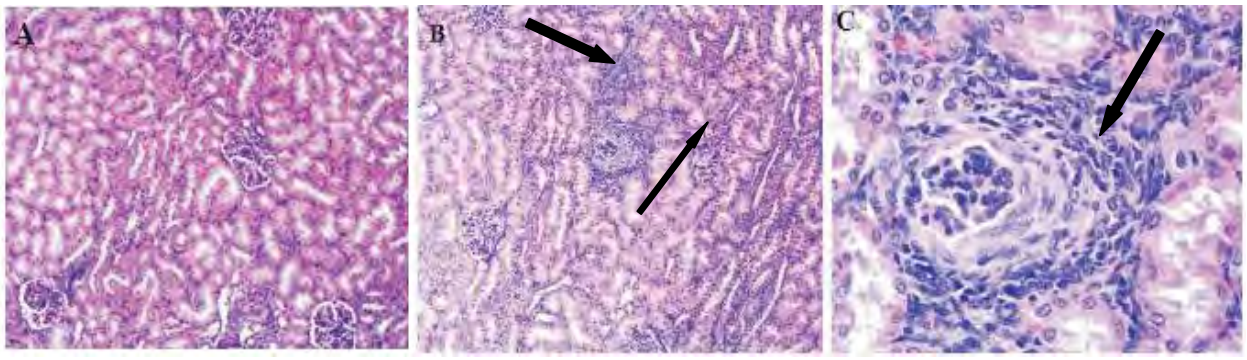

Figure 2. Kidney tissues of control lambs and nano zinc treated group

(A) Kidney of the control group. There are no significant lesions. (B) Kidney of the nano zinc group. Arrows show multifocal interstitial nephritis (infiltration of inflammatory cells). (C) Kidney of the nano zinc group. Arrows show glomerular fibrosis. (H&E).

From the result the revealed that oral administration of nano zinc may cause toxic effects on the liver and kidney in lambs

Wang *et al* (2007) studied on the acute toxicological impact of nano- and zinc oxide powder in liver and kidney adult mice. In this study thirty adult mice weighing about 20–22 g (8-weeks old) were used. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house at 20 ± 28C, 50–70% relative humidity and 12-h light/dark cycle. The animals were provided with commercial rat pellet diet and deionized water ad libitum. After one week acclimation, the mice were randomly divided into three groups. Group one control was given by 1% sodium carboxy methyl cellulose solution instead, group two received 20-nm ZnO and group three received 120-nm ZnO .Each group consisted of five female and five male mice i.e, ten animal each. The mice were administered by 1-, 2-, 3-, 4-, and 5-g/kg body weight 20-and 120-nm ZnO, labeling as N1, N2, N3, N4, N5, and SM1, SM2, SM3, SM4, SM5, respectively. The body weights of mice were recorded before and every two days interval after the administration. Two weeks later, the animals were sacrificed and the blood was obtained from ophthalmic veins.

Biochemical assay of serum

The serum was obtained by centrifugation of the whole blood at 3000 rpm for 15 min.

The serum biochemical levels including lactate dehydrogenase (LDH), alanine aminotransferas (ALT), alkaline phosphatase (ALP), leucine aminopeptidase (LAP), total protein (TP), total cholesterol (TC), tri-glyceride (TG), uric acid (UA), creatinine (CR), serum phosphorus (P), and alpha-hydroxybutyrate dehydrogenase (HBD) were assayed.

Blood-element test and blood coagulation examination

The 0.1 mL of 15-g/L EDTA-Na was pre-added into 1 mL whole blood sample and the anticoagulant blood sample was immediately used for the blood element test within 2 h. The blood-elements, including white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), haematocrit (HCT), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width corpuscular volume (RDW-CV) and blood platelet
(PLT) were assayed.

Histopathological observation

A small piece of liver and kidney were collected and fixed by 10% formalin and then embedded into paraffin, sectioned for 5–6-mm thick, and mounted on the glass microscope slides using standard histopathological techniques. The sections were stained with hematoxylin-eosin and examined by light microscopy

Statistical analysis

The data were expressed as mean ± standard deviation. For statistical analysis, the experimental values were compared to their corresponding control ones. A one-way analysis of variance (ANOVA) in SPSS software (Version 11.0) was used to illustrate the significant difference between the experimental group and the control. The significant difference was considered to be $P < 0.05$.

The result of this investigation showed that body weight (BW) of female mice in N4 and male mice in N1, N5, SM2, SM4 and SM5 groups significantly decreased ($P < 0.05$), The level of LDH in serum is often tested along with ALP and ALT to evaluate whether the liver is damaged or diseased. Further investigation indicates that the significantly elevated LDH and ALT occurred in 1-g/kg body weight treated mice, indicating that liver damage might be induced by low dose of 20-nm ZnO particles. Compared with the 20-nm ZnO treated mice, the median and high dose 120-nm ZnO exposed mice show significantly elevated levels of serum LDH and ALP, suggesting the liver damage. In the study, both significantly elevated LDH and HBD levels were found in 1-g/kg body weight 20-nm ZnO treated mice. Generally, compared with the data of 20-nm ZnO treated mice, the levels of the serum biochemical are higher in 120-nm ZnO treated mice under the same dose administration, suggesting that 120- nm ZnO may cause more severe liver damage than 20-nm ZnO. The elevated RBC and HCT levels were found in N1–N4 and SM3–SM5 group mice. Furthermore, the decreased MCH was found in the 20- and 120-nm ZnO group mice and the decreased

MCHC was observed in N2, N4 and 120-nm ZnO group, histopathological alteration indicate that exposure to 20- and 120-nm ZnO may produce toxic effects on liver and kidney tissues. In liver tissue Edema and degeneration of hepatocytes in the portal area and around central vein of liver the mice exposed to zinc oxide powder on 14-day post Administration were observed, and in renal pathological changes of the mice exposed to zinc oxide powder 5-g/kg body weight 20-nm ZnO group on 14-day post-oral administration show a smack of proteinaceous casts in renal tubule and in 5-g/kg body weight 120-nm ZnO group, the show a spot of proteinaceous casts in renal tubule.

Other study performed by Mahran *et al,* (2011) on the Protective Effect of Zinc on the Histology and Histochemistry of Liver and Kidney in Albino Rat Treated with Cadmium. In this study 60 adult albino rats of both sexes with body weight from 220-250 gm were used. The experiment was conducted to 8 weeks, and the animals were randomly designed into three groups, each of 20 rats. Group one served as control, group two and three served as experimental groups. Rats of experimental group one were injected intraperitonealy with cadmium chloride solution at dose level 0.16 mg/kg of body weight. Rats of experimental group 2 were injected intraperitonealy with both zinc chloride solution at dose level 0.53 mg/kg of body weight. After injection with cadmium chloride in an aforementioned dose. Rats of control group were received injection of the same dose of normal saline. The injections were done into the peritoneum of used rats for eight weeks five times/week. The animals of each group were sacrificed with inhalation of over dose of ether. Small slices of kidney and liver tissue were taken and fixed in 10 % formalin for 24 hours, and were imbedded in paraffin. Five-micron thick sections were routinely stained with hematoxyline and eosin for histological examination under light microscopic observations.

The results of this study showed, liver tissue of a control rat should a normal structure where the liver appeared to decompose of hexagonal or pentagonal lobules with a central veins and peripheral hepatic triads or tetrads embedded in connective tissue. And kidney of control rats**,** had normal renal structure of both (a) cortex which showed a normal structure of; renal glomeruli. The proximal convoluted tubules are lined with typical thick cubic epithelium. The distal convoluted tubules show considerably lower cubic epithelium.

The result of light microscopic examination in the liver tissue of rats treated with Cadmium chloride showed that there were degenerative changes in numerous hepatocytes; the cells were enlarged and had light and foamy cytoplasm filled with numerous vacuole-like spaces. The walls of the blood sinusoids were dilated and presence numerous Kupffer cells. In a few liver zones, the Cadmium chloride induced also hepatocytes necrotic changes which appeared as; a small, pycnotic cellular nucleus with condensed chromatin, lack of nucleolus and strongly acidophilic cytoplasm. Mononuclear cell Infiltrates were also noted in hepatic areas. And the kidney tissue of rats treated with Cadmium chloride only showed that there were many areas of tubular damages ranged from mild to severe in the kidney were observed in all treatment animals. These renal damages appeared as hypertrophy and degeneration of epithelia of renal tubules with distinct of mononuclear cells infiltration. A few renal tubules showed single epithelial cells

desquamated to their lumen. Also some vascular glomeruli were apparently enlarged, tightly filling the Bowman's capsule with absence of the capsular spaces was observed. Moreover, hyperemia of the kidney vessels was observed.

Zinc chloride in combination with Cadmium chloride caused reduction of toxic effects on Cadmium chloride toxicity on the liver tissue. In which an absence of nucleus fragmentation and a decrease in the sinusoidal dilation, necrosis of some hepatocytes, mononuclear cell infiltrations were observed. In fact they noticed the presence of rare inflammatory sites in the sinusoids and some hepatocytes with light cytoplasm. Light microscopic examination also revealed a positive correlation between Zinc chloride and Cadmium chloride in the kidney tissues with marked reduction of the toxic effect on the kidney. However, some toxic effects of Cadmium chloride, as mild hyperemia in the kidney vessels, some degenerative changes in the tubular epithelium and cystic dilatation were observed.

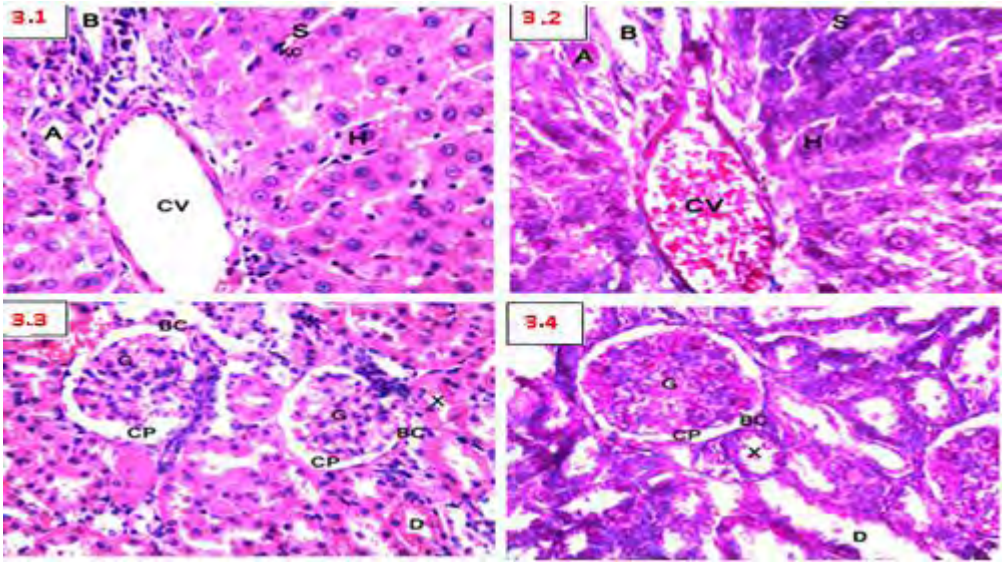

Figure 3.Light photomicrography of liver and kidney of a control rat.

Figure 3.1 and 3.2: Light photomicrography of liver of a control rat: It is composed of lobules which are roughly hexagonal in shape, with portal triads at the vertices and a central vein (CV) in the middle. Within each lobule, hepatocytes (H) are arranged into hepatic cords running radiantly from the central vein and are separated by adjacent blood sinusoids (S) containing Kupffer cells. N.B; Bile duct (B), Hepatic artery (A).

Figure 3.3 and 3.4: Light photomicrography of Kidney (cortical part) of a control rat. The renal glomeruli (G) show normal structure and the proximal (X) are lined with typical thick cubic

epithelium and distal (D) convoluted tubules are lined with the relatively low simple cubic epithelium. Organization of the glomeruli and a flat epithelium lining the glomerular capsule (BC) with distinct capsular space (CP) can be seen.

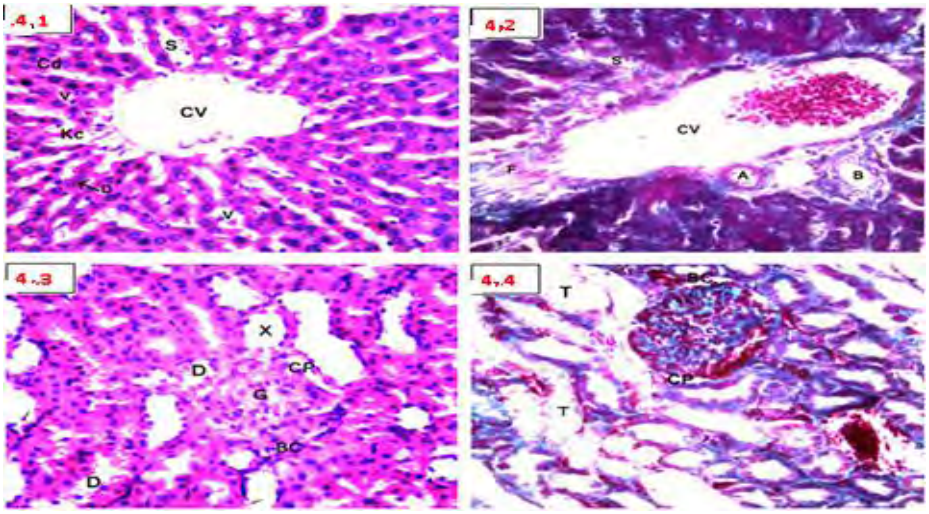

Figure 4. Light photomicrography of liver and kidney of a rat after eight weeks of exposure to CdCl2.

Figure 4.1 and 4.2: Light photomicrography of liver of a rat after eight weeks of exposure to CdCl2. The trabecular structure of the liver is blurred. The hepatocyte cytoplasm is light, foamy and filled with vacuoles (V); cell sizes are enlarged, nuclear chromatin is more condensed (Cd). Necrosis of single hepatocytes and the nuclei are contracted, pycnotic with condensed chromatin (D arrow), cytoplasm is strongly acidophilic. Accumulation of mononuclear cells in the vicinity of sinusoids (S).The sinusoid walls shows numerous Kupffer cells (Kc).

Figure 4.3 and 4.4: Light photomicrography of Kidney of rat after eight weeks of exposure to Cd Cl2, showing a vascular glomeruli (G) are enlarged, tightly filling the glomerular capsular space (CP),with flat epithelium lining the Bowman's capsule (BC) Some cells of the proximal (X) and distal (D) convoluted tubular epithelium show features of edema. Capillaries are filled with blood cells; some tubules contain single desquamated cells (black lines).

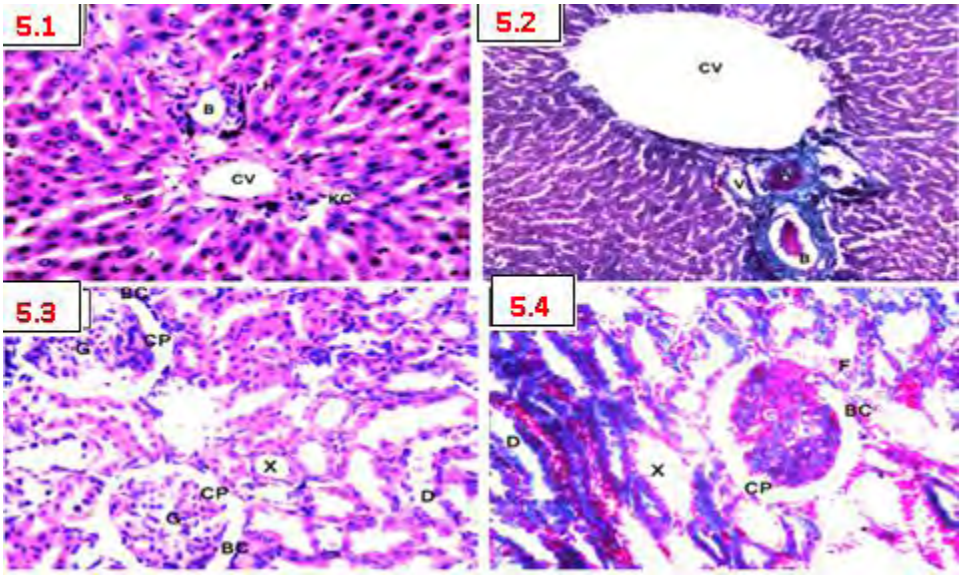

Figure 5: Light photomicrography of liver and kidney of rat after eight weeks of exposure to Cd Cl2 and Zncl2.

Figure 5.1 and 5.2: Light photomicrography of liver of rat after eight weeks of exposure to Cd Cl2 and Zncl2.The liver organization appears normal with decreased widening of blood sinusoid (S), less fragmentation of hepatocytes nuclei, less lighting of the cytoplasm and decrease the mononuclear cells infiltration in the Vicinity of portal system mainly around the central vein (CV) and bile duct (B)**.**

Figure 5.3 and 5.4: Light photomicrography of Kidney of rat after eight weeks of exposure to CdCl2 and ZnCl2 .showing an decreased in the vasculature of the renal glomeruli (G), appearance of the glomerular capsular space (CP).Decrease the edema **of** both the proximal (X), and distal (D) convoluted tubular epithelium. Lack of the fibroses in the Bowman's capsule (BC)**.**

Also other study investigated by Abdel *et al*, (2011) on the toxic effects of zinc on the liver structure of Nile tilapia, Oreochromis niloticus fish. In this study Two hundred and forty acclimated fish weighted 24.30± 2.85 g were used. Fishs divided into two groups; the first group was exposed to 2, 4 and 6 mg/L of Zinc chloride which represent 0.25, 0.50 and 0.75 of Zn LC50 respectively for one week (short exposure period) in addition to 0.00 LC50 as a control. 80 L glass aquaria (100 × 50 × 40 cm) were used with three replicates for each concentration. The second group was exposed to the same concentrations of ZnCl2 with the same replicates for 4 weeks (long exposure period). Fish were fed twice daily at a rate of 2% of body weight with 32% crude protein diet.

Estimation of LC50

To determine the 96 h LC50 of exposure, seventy fish were randomly distributed in seven small aquaria (40 L). The fish were exposed to various concentrations (2, 4, 6, 8, 10, 12 and 14 mg/L) of zinc chloride (ZnCl2) which previously dissolved in distilled water and added to the aquaria. Fish were exposure to the above concentrations for 96 h. Mortality in each aquarium was recorded daily and removed. To find out the survival time in each concentration of ZnCl2 observations were recorded, and then the LC50 value was calculated from the regression line drawn The 96 h LC50 was 8 mg /L.

Histological examination

Liver samples of the control and treated fish were fixed in 10% neutral- buffered formalin, and then the samples were processed for routine wax histological evaluation (dehydrated and embedded in paraffin). Sections of 5μm were done and stained with hematoxylin and eosin.

The result of control fishes liver tissue generally exhibited a normal mural architecture with polygonal shaped hepatocytes, having a large spherical nucleolus and variable amount of dispersed and peripheral heterochromatin. Hepatocytes were located among blood capillaries called sinusoids forming cord-like structure known as hepatic cell cords. The lumen of sinusoids contained mainly erythrocytes. Kupffer cells were found to rest on the luminal surface of the sinusoids endothelium.

Short exposure period (acute exposure)

Light microscopic study of the fish liver exposed to Zinc for one week showed several changes. The liver cells were degenerated; the normal architecture of liver was markedly disorganized. Hypertrophy of hepatocytes which had pycnotic nuclei was quite evident in liver of fish exposed

to all metal concentrations from 2 to 6 mg/L of LC50 zinc chloride. In addition, dilated sinusoids with congestion were noticed, the intra hepatic blood vessels were dilated and congested with blood cells in liver of fish exposed to metal concentrations 4 and 6 mg/L of LC50 zinc chloride .Moreover, a marked increase in numbers of Kupffer cells was observed in liver of fish exposed to metal concentration of 6 mg/L zinc chloride.

Long exposure period (chronic exposure)

Liver of fish exposed to effluent levels for 4 weeks revealed varying degrees of histopathological alteration due to damaging of cell structure with increase of hypertrophied hepatocytes in liver of fish exposed to all Zinc concentrations. The nuclei displayed pleomorphism with peripheral nucleoli in liver of fish exposed to metal concentration of 4 mg/L zinc chloride. Furthermore, the hepatocytes exhibited focal necrosis resulting in complete disintegration of cellular components as evidenced by the presence of darkly stained eosinophilic debris in liver of fish exposed to metal concentration of 6 mg/l zinc chloride. In all metal concentrations (2 to 6 mg/L of LC50 zinc) there was extensive dilation of sinusoids with blood congestion. In some places hypertrophy and hyperplasia of bill duct cells and sign of blood vessels fibrosis were noted at concentration of 6 mg/L zinc.

Firas *et al*, (2012) conducted a research to investigate effect of zinc on the hematological and the histological effect on heart, kidney and liver of the domestic ducks. In this study sixteen local duck (*anaspater hycous)* were used. The animals were divided randomly into two groups of eight animals each. The animals of the first group was the control group and the second group was experimental group and dosed 100mg/kg (B.W) body weight zinc sulphate for four weeks. The average weight of the animals was 1.250-1.750 kgm and of 12 months age. At the end of the experiment, 5ml blood was taken from each animal in to 2 test tubes (one with EDTA and the other without EDTA) .Blood with EDTA was used to measure RBC with help of Hematocytometer concentration of Hb and PCV were measured. Blood without EDTA was centrifuge at 1500 rpm for 20 minutes to separated serum. Serum cholesterol and total protein were measured and Date was analyzed statistically used analysis of variance.

Histologicaly, the demanded organs such as heart, kidney, liver and pancreas were picked and put in formalin 10%. After three days dehydration process was done by the use of ascending

concentrations of ethanol (70%, 80%, 90%, and 100%) whilst the last two concentrations were repeated to ensure a good dehydration process. The xylene was used to clear the organs. The specimens were soaked and embedded with paraffin wax then they were cut by the use of microtome at a thickness of 7 degrees. The samples were stained with Hematoxylin &Eosin stain. With this set up the following histological result were obtained and only result of liver and kidney are provided here.

Table 3: levels of RBC, PCV, Hb, MCV, MCH, and MCHC in control and experimental animals

	R.B.C *(10)6	**P.C.V %**	**Hb mg/dl**	**MCV**	**MCH**	**MCHC**
Control G1	**2.510 ± 0,245 b**	**46.875 ± 6.664 A**	**18.525 ± 0.656 A**	**186.786 ± 20.744 A**	**74.377± 7.162 A**	**40.142 ± 5.164 A**
Experimental 100mg/kg G2	**3.443 ± 0.519 A**	**50.250 ± 2.7 A**	**17.000± 0.709 A**	**149.545± 28.543 B**	**53.136 ± 8.975 B**	**36.664 ± 1.369 B**

Result are expressed as mean ± S.D

***** Represents significant difference at $p \leq 0.05$

Table 4: levels of Cholesterol, GPT and GOT in control (G1) and experimental group (G2) animals

	Cholesterol mg/dl	Total Protein Mg/dl	GPT i.u	GOT i.u
G1	188.875 ± 4.911 B	5.787 ± 0.584 A	30.375 ± 1.685 B	42.375 ± 2.875 B
G2	200.125± 7.698 A	5.662 ±0.647 A	33.187± 2.852 A	57.875 ± 3.482 A

Result are expressed as mean ± S.D

***** Represents significant difference at $p \leq 0.05$

Histological results as it's shown in the following pictures where the changes which occurred after offering zinc are seen:

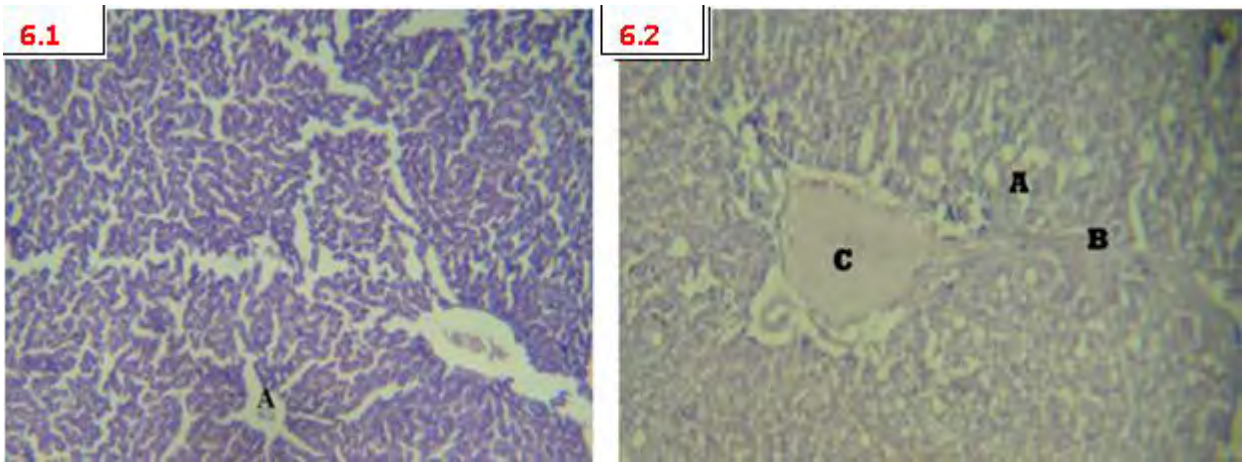

Figure 6. Histological section of liver control liver and treated with zinc sulphate

Figure 6.1 transverse section in control liver show the central vein (A) central vein H&E. And Fig 6.2 Histological section of liver of second group shows a fatty degeneration in the hepatic cells (A fibers accumulation in intralobular trabeculi (B) dilation in the blood vessel(C). H&E stain

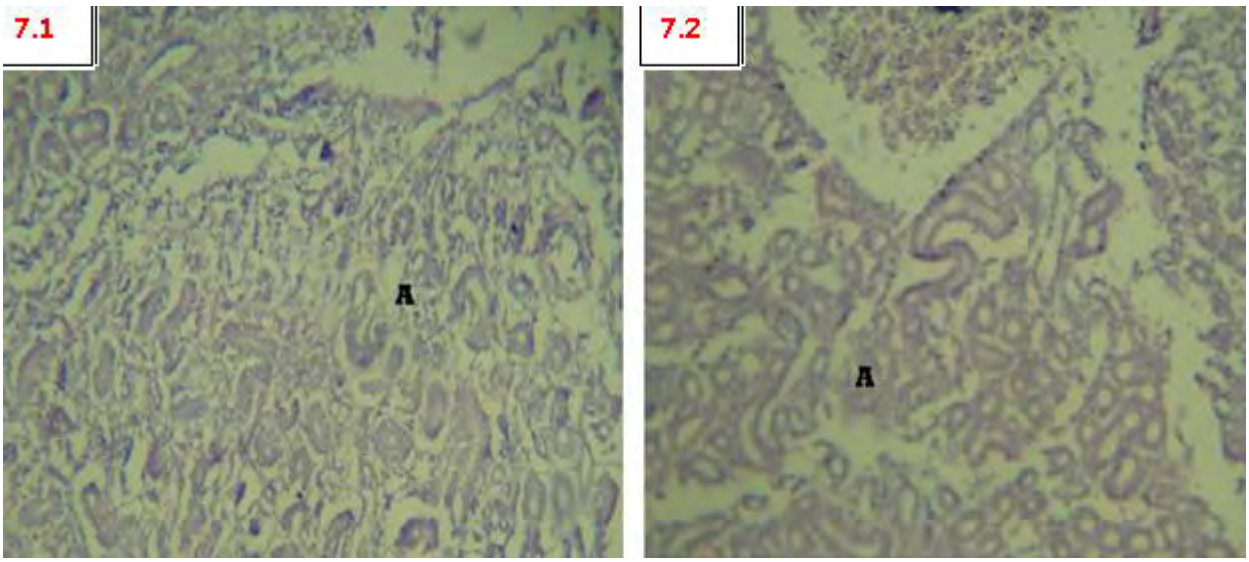

Figure 7.Histological section of kidney treated with zinc sulphate

.Figure (7.1) Histological section of kidney shows degeneration in renal tubules lining cells (A). H&E stain**.** And Fig (7.2) Histological section of kidney (G2) shows hydropic degeneration in renal tubules lining cells (A). H&E stain.

Other study performed by Gathwan *et al*, (2012) on the toxic effects of zinc and Cadmium on the liver of male mice. In this study 24 Adult male mice, weighing 32-34gm of Blab /c, 70 days old were used. The animals were divided into four groups each group having 6 mice. Group one is control group on normal diet and water the rest three groups are experiment groups. The experimental animals were treated orally with different doses of cadmium chloride daily for 21 days, as follows (1mg/kg body wt., 5mg/kg body wt., and 10 mg/kg body wt) and zinc chloride daily for 21 days, as follows (1mg/kg body wt., 5mg/kg body wt., and 8 mg/kg body wt . The body weight and liver weight from treated groups was taken along with control after 21 days.

The liver was cut into small pieces and fixed in Bouins fixative. Histological examination of liver was carried out by standard histological techniques. Sections of 7 µm thickness were cut and stained with hematoxylin: eosin.

Statistical analysis: Results are reported as mean + SE. In experiments where the cadmium chloride and zinc chloride doses were varied, data was analyzed by using student's "t" test.

The result of this study showed a signs of metal toxicity were observed with 10 mg/ kg body wt., and 8 mg/kg body Weight of administration of cadmium chloride and zinc chloride These included Shivering, salivation and lacrimation.

The intake of feed and water by treated mice reduced as compared to control. Moreover, the decrease was dose dependent. The liver weight and body weight show significant decrease with increase of doses. (Fig.8.Table 5 and 6).

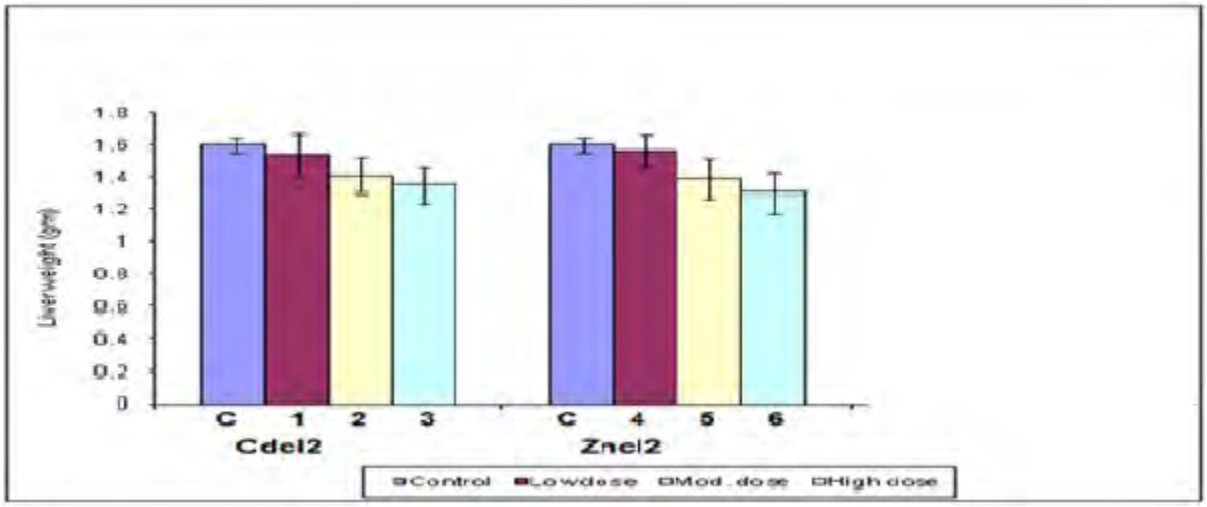

Figure 8: Effect of CdCl2 on liver weight of male mice (values represent mean ± S.D., n=6 in each group.

Table 5: Effect of ZnCl2 on body weight of male mice

Group	Initial Body Weight (gm)	Final Body Weight (gm)	Percentage Change
Control	31.6±2.1	34.36±3.08	8.34
Low dose	32.8±2.9	31.56±1.4*	-3.42
Mod. Dose	31.2±3.03	29.4±3.0**	-5.76
High dose	32.0±2.1	28.3±2.5***	-11.56

Value represents mean ± S.D., n=5 in each group

The P-value was calculated between the test group and control group.

* Non-significant different from the control value $p>0.05$.

** Significant different from the control value $p<0.05$.

*** Highly significant different from the control value $p<0.001$

Histological result of liver after various doses of Cadmium chloride and Zinc chloride treatment showed marked alteration.

The metal treatment caused marked changes in liver such as swelling and massive fatty degeneration in hepatocytes and large vacuoles in cytoplasm. Cytoplasm of hepatocytes showed vacuoles and nuclei with pycontic and staining affinity of nucleus was comparatively poor, due to damage of the hepatic cells after treatment with Cadmium chloride and Zinc chloride. The damage of hepatic cells increase with increase of dose was observed. Apoptosis was observed at 10 mg/kg body wt., Cadmium chloride and 8 mg/kg body wt. Zinc chloride administration.

Pallavi and Neera (2004) studied the Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc in kidney of fish Channa punctatus. Live specimen of freshwater fish, C. punctatus were collected from water bodies and acclimatized for two weeks under laboratory conditions. On the basis of 96 hr LC50 of zinc for C. punctatus three sub-lethal concentrations i.e. 10 mg/l, 15mg/l and 25 mg/l were selected for the present study. Fish were exposed to these concentrations of zinc for 15 days LC50 value was calculated by developing a regression equation. Zinc was given in the form of zinc sulphate dissolved in distilled water. Group I served as control. Observations were made on 8th, 10th and 15th day. After 15 days these fish were transferred to normal tap water and recovery responses were observed for 15 more days. Recovery responses were observed on 8th, 10th and 15th day. At the end of specified periods of experimental exposure, the kidney tissue was weighed as 30 mg for each sample and digested in a diacidic solution . Digested samples were analyzed by atomic absorption spectrophotometer in air acetylene flame at 213 E for estimation of the zinc content. Data obtained was subjected to statistical analysis. The difference between control group and experimental groups were analyzed with the help of student't'.

In the present study zinc concentration in kidney of treated groups showed statistically significant increase ($p<0.01$) in all groups and at all intervals in comparison to control (Table 7). Zinc concentration in treated groups increased with an increase in dose and duration of treatment. Statistical analysis by ANOVA also shows that uptake of zinc is significantly ($p<0.01$) influenced both by dose and duration of the experiment. The dose levels of zinc administrated in the present experiment appear to affect the detoxication mechanism within the kidney thereby retarding metal elimination and enhancing its accumulation.

Results of recovery phase still show highly significant ($p< 0.01$) retention of zinc by the kidneys of C. punctatus in comparison to control (Table 8). ANOVA also show that the variations between treated groups and between intervals are highly significant ($p< 0.01$). However, a mild trend towards elimination of zinc could be observed in all post exposure recovery groups with the passage of time.

Slow elimination of zinc in the present study indicates that the stress of pre-exposure probably alters certain basic biodegradation mechanisms and that a longer period may be required for zinc to fall within normal levels. Thus accumulation of zinc is much quicker than its elimination as retention of zinc could be noted up to 15 days.

Table 6: Zinc accumulation by the kidney (/g) of fish C. punctatus subsequent to exposed to sub-lethal concentrations of zinc

	Day 8	Day 10	Day 15
Group 1 (Control)	108.88 ± 1.67	107.94 ± 1.69	110.10 ± 1.30
Group II (10 mg/l)	207.66 ± 0.98**	225.77 ± 0.56**	269.88 ± 0.88**
Group III (15 mg/l)	252.23 ± 0.97**	264 ± 1.05**	293.33 ± 1.01**
Group IV (25 mg/l)	316.94 ± 0.96**	328.16 ± 1.43**	359.75 ± 1.89**

**Significant at $p < 0.01$

Table 7: Zinc concentration in the kidney (Eg/g) of fish C. punctatus pre-exposed to sub-lethal concentrations of zinc.

	Day 8	Day 10	Day 15
Group 1 (Control)	105.16 ± 1.63	109.32 ± 0.91	102. ± 1.12
Group II (10 mg/l)	245.83 ± 0.93**	235.38 ± 0.73**	219.55 ± 0.75**
Group III (15 mg/l)	273.55 ± 0.80**	254.10 ± 0.87**	236.38 ± 0.83**
Group IV (25 mg/l)	332.99 ± 0.91**	297.27 ± 0.72**	268.49 ± 0.71**

**Significant at $p<0.01$

Histopathological changes in the kidney were observed at day 8 and continued till the termination of the experiment. Renal tubules became highly expanded; their epithelial lining was distinctly separated from the tubular cells. Some renal tubules were characterized by loss of cellular integrity. Dilation, edemas and hypertrophied nuclei of renal tubules are also noted. Glomeruli show vacuolization and disorganized blood capillaries. Necrosis and pyknotic nuclei can be observed in mesenchymal tissue. Damage becomes more pronounced by day 15 and is more severe in groups III and IV. The study also indicates that after withdrawal of zinc, recovery is not spontaneous, it very slow but progressive. It can be inferred that fishes are unable to overcome the stress of per-treatment within 15 days, and duration longer than 15 days is required for normalizing the tissue damage and elimination of accumulated zinc.

Other study conducted by Oznur et al (2012) on the effect of zinc on ethanol-induced oxidative stress in rat liver. In this study thirty nine, six-month-old, male, Wistar rats (250-300g) were used. The animals were housed in a room maintained at 24° C with a 12-hour light-dark cycle. The animals were given stand art rat chow and water ad libitum. After a quarantine period of 5 days, rats were randomly divided into four groups maintained for 13 days as follows: Control group (n=10): Animals were fed standard was injected intraperitoneally (i.p.) with 0.9% saline. Ethanol group (n=10): Ethanol was given intraperitoneally (i.p.) at a dose of 2g/kg/day. Zinc group (n=10): Zinc sulphate (ZnSO4.7H2O) was administrated at a dose of 7 mg kg/ day orally. ZnSO4.7H2O was dissolved in 0.5 ml distilled water. Ethanol+Zinc group (n=9): Zinc sulphate was administrated orally at a dose of 7 mg kg/day 10 minutes after the ethanol was injected i.p. at a dose of 2g kg/day. On the 13th day, rats were euthanized with intravenous 40 mg/kg ketamine hydrochloride and 2.5 mg/kg xylazine injection. Liver tissues were quickly removed, washed with NaCl, immediately frozen in liquid nitrogen, and were kept at -80 °C until studied.

Histological Evaluation

Liver tissues of all groups were fixed in phosphate-buffer containing 2.5% gluteraldehyde for 2-3 hours. Then they were post fixed in 1% osmium tetra oxide (OsO4) and dehydrated in a series of graded alcohols (50, 60, 70, 80, 90, 96 and 100% ethanol). After passing through propylene oxide, the specimens were embedded in Araldyte CY 212, DDSA (2-dodecenyl succinic anhydride), BDMA (benzyldimethyl amine) and dibutylphitalate. Semithin sections were cut and stained with toluidin blue and examined with Olympus light microscope.

Histological Results

In the ethanol group; dilatation in sinusoids, lipid granulles in hepatocyte cytoplasm, vacuolization in sinusoidal endothelial cells, increase in fibrotic tissue between hepotocytes and apoptic appearence in some of the hepatocyte nucleuses and dilatation in biliary ducts were observed. In contrast, in the ethanol zinc group, the lipid granules were not observed. Both the dilatation in sinusoids and in biliary ducts, fibrotic tissues between hepatocytes, vacuolisation in sinusoidal endothelial cells was decreased. In the zinc group; although the general structure seemed to be normal and it observed minimal dilatation in sinusoids and vacuolization in endothelial cell cytoplasm. It was observed that the most prominent changes were seen in the ethanol group following the ethanol plus zinc group.

3. Discussion

Since the recognition of zinc as an essential nutrient, many researchers have studied its role in the prevention and treatment of toxic metals such as aluminum, cadmium, as well as organic solvent such as ethanol. The research publications reviewed and analyzed here are on the toxic and protective effect of zinc against aluminum, cadmium and ethanol toxicity on histology of liver and kidney.

Gawish (2005) and Mahran *et al*., (2011), reported that hepatic and renal degeneration was observed in rats exposed to different doses of Aluminum and Cadmium, at 50mg /kg/day dose of hydrated Aluminum sulphate and 0.16 mg dose Cadmium chloride/kg respectively. Liver tissue daily treated with Al sulphate and Cadmium chloride showed enlarged cells and cytoplasm filled with numerous vacuole-like spaces. The walls of the blood sinusoids were dilated and hepatocytes necrotic changes, pycnotic cellular nucleus with condensed chromatin, lack of nucleolus and strongly acidophilic cytoplasm. Fragmented nuclei with mononuclear cell Infiltrates were appeared. Zinc supplementation after Aluminum and Cadmium, showed improvement in liver tissue in which hepatocytes appeared with their normal structures, an absence of nucleus fragmentation and a decrease in the sinusoidal dilation. In fact both investigators noticed the presence of rare inflammatory sites in the sinusoids and some hepatocytes in liver tissue, and kidney tissues also showed less shrinked corpuscle with marked reduction of the toxic. These results were compatible with the findings of Oznur *et al* (2012) who reported that there is presence of histopathological changes in liver tissue of ethanol treated rats at a dose of 2g/kg/day. However oral Zinc supplementation, at a dose of 7 mg kg/day, showed improvement in liver tissue in which lipid granules were not observed. In addition both the dilatation in sinusoids and in biliary ducts, fibrotic tissues between hepatocytes, vacuolization in sinusoidal endothelial cells were decreased. This is because all these studies used same animal model i.e rats, similar doses and duration of experiment .Kidney tissue after daily treated with Aluminum sulphate and Cadmium chloride at similar doses also showed shrinkage in the corpuscles, congestion of renal corpuscles, and renal tubules cells loosed their normal shape indicating distortion in their structures. Also some vascular glomeruli were apparently enlarged, tightly filling the Bowman's capsule with absence of the capsular spaces and that, with a concomitant administration of Zinc to Aluminum and Cadmium, Zinc improved amelioration of kidney tissue and it showed less shrinked corpuscle with marked reduction of the toxic effects.

The results of all the above protective effects of zinc against toxic heavy metals were in agreement with Hafiez *et al.*, (1989 & 1990), and Mansour *et al., (*1989). Zinc is known to play a special role in protecting the tissues of liver, kidney, brain and testis against free radicals cited by Gawish (2005). In contradiction to the reported protective effect of zinc, some studies reported zinc toxicity as a result of zinc and cadmium combination toxicities in liver tissue. This result may be due to different animal model used, different doses and duration of experiment. Among those reports, a study supported by Gathwan et al, (2012) studied on the toxic effects of zinc and Cadmium on the liver of male mice .The animals were treated orally with different doses of cadmium chloride daily for 21 days, as follows (1mg/kg body wt., 5mg/kg body wt., and 10 mg/kg body wt) and zinc chloride daily for 21 days, as follows (1mg/kg body wt., 5mg/kg body wt., and 8 mg/kg body wt showed that marked changes in liver such as swelling ,massive fatty degeneration in hepatocytes nuclei with pycontic and large vacuoles in cytoplasm., due to damage of the hepatic cells after treatment with cadmium chloride and zinc chloride. The damage of hepatic cells increase with increase of dose was observed. These results also are in agreement with Morsey, M.G and Protasowicki.M, (1990). This is because all these studies used same animal model, similar doses and duration of experiment.

Firas *et al. (*2012) also done experimental study to assess the effect of zinc sulphate exposure associated with hematological and histological effect on kidney and liver of the ducks. The experiment was carried out on 16 domestic ducks and the animals was 1.250-1.750 kgm average weight and of 12 months age. After the experiment animal were dosed 100mg/kg(B.W) body weight zinc sulphate for four weeks, the liver showed fatty degeneration in the hepatic cells, fibers accumulation in intralobular trabeculi, dilation in the blood vessel (fig, 6.2) compared to control. This result agree with Gathwan et al, (2012) accordingly after treatment Zinc chloride at1mg/kg body wt., 5mg/kg body wt., and 8 mg/kg body wt dose level liver tissue showed degenerative changes. In which swelling and massive fatty degeneration in hepatocytes, dilation in the blood vessel were observed; whereas histological section of kidney shows degeneration in renal tubules lining cells and degeneration in renal tubules lining cells (Fig, 7.2). This result also compatible with Pallavi and Neera (2004) after increase zinc concentration as zinc sulphate in the kidney of fish treated group showed renal tubules loss of cellular integrity, dilation, edema and hypertrophied nuclei of renal tubules with necrosis and pyknotic nuclei observed in mesenchymal tissue. Najafzadeh *et al*, (2013), also in line with the result of Gathwan et al,

(2012) and Pallavi and Neera (2004) accordingly Kidney of the nano zinc group at dose of 20mg/kg body weight on 25days oral administration Showed multifocal interstitial nephritis (infiltration of inflammatory cells) and glomerular fibrosis.

Abdel-Warith, *et al*, (2011) assessed on the toxic effects of zinc (Zn) on the liver structure of Nile tilapia, Oreochromis niloticus fish at different exposure periods. and after fish exposed to 2, 4 and 6 mg/L of ZnCl2 which represent 0.25, 0.50 and 0.75 of Zn LC50 respectively for one week (short exposure period); while the second group was exposed to the same concentrations of ZnCl2 with the same replicates for 4 weeks (long exposure period). Light microscopic study of the fish liver exposed to Zn for one week (Short exposure period and 4 weeks (long exposure period) showed several changes. The liver cells were degenerated; the normal architecture of liver was markedly disorganized. Hypertrophy of hepatocytes which had pycnotic nuclei was quite evident in liver of fish exposed to all metal concentrations from 2 to 6 mg/L of LC50 zinc chloride. In addition, dilated sinusoids with congestion were noticed, the intra hepatic blood vessels were dilated and congested with blood cells .Moreover, a marked increase in numbers of Kupffer cells. Furthermore, the hepatocytes exhibited focal necrosis resulting in complete disintegration of cellular components as evidenced by the presence of darkly stained eosinophilic debris was observed. thus result were also in line with Najafzadeh *et al*, (2013),who reported that oral administration of zinc oxide nano-particles to lambs by dose 20mg/kg body weights daily for 25 day shows eosinophili necrosis and cell swelling hepatocytes in Liver tissue. The method used in their studies, animal model, duration of experiment and duration of doses are different. The only similarity of all investigators used Zn and zinc compound as zinc chloride, zinc oxide and zinc sulphate to study the toxic effect of zinc. However all investigators observed that liver and kidney tissues damage increase with increase of doses and exposure time of zinc and zinc compounds.

Liver and kidney are more closely related to blood on their function, it is customary to discuss and analyze hematological result and blood parameter in order to get information on the efficiency of the function of the two organs after exposure.

Accordingly, the hematological change induced by zinc exposure was described by Firas et al, (2012) .In this study zinc sulphate was exposed to domestic ducks by dose of 100mg/kg (B.W) for four weeks to experimental group to compare with controls. Before and after exposure, hematological result like, Hg concentration, PCV percentage and RBCs count were examined.

Accordingly a significant increase in RBC. And non significant decreases in Hb of the experimental group comparing with those of control group were observed (Table 3). The Significant increase of RBC count due to decrease in the hemoglobin concentration which produce decrease in oxygen transport from lungs to body tissue (Hypoxia) , the body in the case of hypoxia will stimulate the bone marrow to produce new RBC to maintain the oxygen level in the blood and body tissue (Chunn,1973),cited by Firas et al, (2012). The MCV, MCH and MCHC of experimental group were significantly deceased comparing with those of control group. This result is agreed with Wang *et al* (2007) who reported that oral administration of 5-g/kg body weight ZnO nanoparticle, elevated RBC and HCT levels were found in experimental animal group (mice) Furthermore, the level of MCH, MCHC of experimental group were significantly deceased comparing with those of control group. The decrease in M.C.V., MCH, and M.C.H.C. is associated with anemia. Some of the previous studies also concluded that excessive dietary zinc in animals could induce deficiencies of copper and iron and then produce growth retardation and anemia (Torrance and Fulton 1987; Latimer et al. 1989; Hoffman et al. 1988; Llober et al. 1988; Hein 2003).cited by Wang *et al* (2007).

The blood biochemical tests are frequently used in diagnosis diseases of liver, kidney. The level of LDH in serum is often tested along with ALP and ALT to evaluate whether the liver is damaged or diseased. When the liver is in dysfunction, the levels of the above serum enzymes will raise, (Kellerman 1995). Accordingly Wang .*et al* reported that median and high dose of ZnO nanoparticle exposed mice show significantly elevated levels of serum LDH and ALP, suggesting the liver damage. The level of blood BUN and creatinine are also good indicators for renal function. If kidney function falls the BUN and creatinine levels will rise. Thus, the significantly increased creatinine level in the nano zinc group in this study suggested that the renal dysfunction be most likely caused by nano zinc administration to lambs (Najafzadeh H, et al. (2013)).this result also agree with Llobet *et al* (1988) reported that, the concentrations of urea and creatinine in plasma significantly increased after high-dose exposure to zinc acetate dihydrated in drinking water.

4. Conclusion

Different scientific studies analyzed and reviewed in the present paper have reported that toxic metals such as Al, Cd, as well as organic solvent such as ethanol cause histopathological changes in liver and kidney. These changes include hepatocyte degeneration, nuclear pycnosis, cellular swelling, and congestion of blood vessels appeared in liver tissue. Degeneration in the renal cells, renal tubules became highly expanded and their epithelial lining was distinctly separated from the tubular cells glomeruli show vacuolization and disorganized blood capillaries was observed in kidney tissue. Zinc administration together with Aluminum, Cadmium, and ethanol had protective effect against Aluminum, Cadmium toxicity in liver and kidney tissues and toxicity ethanol liver tissue.

However, in some studies reviewed here, Zinc was shown to have toxic effects on liver and kidney tissues at higher concentration and with increased duration of treatment. The major effects included hepatocyte degeneration, nuclear pyknosis, cellular swelling, and congestion of blood vessels appeared in liver tissue. Degeneration in the renal cells, renal tubules became highly expanded and their epithelial lining was distinctly separated from the tubular cells glomeruli showed vacuolization and disorganized blood capillaries was observed in kidney tissue. In addition to this significantly increased in level of RBC, BUN and creatinine in serum biochemistry. In contrast blood level of MCH, MCHC and Hb are significantly decreased. This controversy result could be due to dose level, model animal used, and duration of the experiment, age and weight of the animal used. In general, all the investigations reviewed here did not revealed similar results. Therefore, repeating or conducting similar investigations on protective effects of Zinc and Zinc compounds on heavy metal toxicities, using increased sample sizes may be required for conformation and determining optimal dose.

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