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TOXICO PATHOLOGICAL STUDY OF COPPER SULFATE MODULATE BY ZINC OXIDE AND *CORIANDRUM SATIVUM* PLANT TREATMENT IN MICE

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Abstract

This experiment was designed in order to study the degree of toxic pathological effects of copper sulfate associated with zinc oxide and extract of *Coriandrum sativum* treatment in mice. We used 110 mice conducted on eleven groups of 10 mice 1st group treated with distilled water as control negative, 2nd group treated with copper sulfate and then treated with distilled water as control positive, 3rd group treated with copper sulfate only, 4th group treated with low concentration copper sulfate with zinc oxide, 5th group treated with high concentration of copper sulfate with zinc oxide, 6th group traded with low concentration of copper sulfate and *Coriandrum sativum*,7th group treated with high concentration of copper sulfate and zinc oxide and *Coriandrum sativum*, 8th group treated with copper sulfate and *Coriandrum sativum*, 9th group treated with copper sulfate at high concentration and *Coriandrum sativum*, 10th group treated with zinc oxide only, 11th group treated with *Coriandrum sativum* only. Each treated period for each groups were 8 weeks. Early patho-toxicological changes were: Congestion, edema, degenerations followed by necrosis in brain, atrophy and paleness of liver and kidneys. Hyperkeratosis within stomach wall. Suppurative broncho-pneumonia. increased of apoptosis for hepatocyte with increase of inclusion bodies in epithelial layer of renal tubules, present for renal cast, splenic white pulp depletion. These finding prefill toxicity by copper sulfate in mice but at the same time, treatment of zinc oxide and *Coriandrum sativum* or both acts as protection against toxicity of copper sulfate .

***Key words*:** Copper, toxicity, *Coriandrum sativum*.

Introduction

Copper is an essential trace element that is widely distributed in tissues of animal and plant (US.AF., 1990 and Institute of Medicine, 2001). Copper is very important for iron metabolism, collagen and elastin system, numerous metalloproteinase enzymes and non-metalloproteinase enzymes in animal which require copper to be biological active, recently it has been shown that cu is one of the key trace mineral required for an effective immune response (Harrison *et al*., 2000) .Copper is absorbed, transported, distributed, stored, and excreted in the body according to complex homeostatic processes which ensure a constant and sufficient supply of the micronutrient while simultaneously avoiding excess levels (Stern, (2007, 2010). The recommended dietary intake for copper is 1.5-3.0 mg/day for adults; 0.7-2.5 mg/day for children; 0.4-0.7 mg/day for infants (US.AF., 1990). But Ingestion large doses less than one gram (637 mg/kg copper) cause's copper poisoning (Chuttani *et al*., 1965). Copper exposure, liver and other organ meats, seafood, nuts and seeds (including whole grains) are good sources of dietary copper (Institute of Medicine, 2001 and Harrison, 2000). Copper is released into the environment by mining, farming, and manufacturing operations and through waste water releases into rivers and lakes. Also released from natural sources, like volcanoes, wind blown dusts, decaying vegetation, and forest fires (ATSDR, 2004). Another source of copper is found in surface water, ground water, sea water and drinking water (ATSDR, 2004). The suggested safe level of copper in drinking water for humans varies depending on the source, but tends to be pegged at 2.0 mg/L (Nauja *et al*., 2008). In humans acute copper poisoning has occurred through contamination of beverage through storage in containers copper containing as well as contamination water (ATSDR, 2004). While ingested or cutaneous absorption of adequate amount of copper salt is able to produce acute gastro enteric poisoning, the usual poisoning in domestic animal is a chronic disorder, when copper stored in the liver reaches a critical level (Expert Group, 2002). Copper sulfate is the most common copper salt; it is used chief for agriculture purposes as a pesticide and in leather industry (ACGIH, 1986). A dose response effect to following ingestion is difficult to define but approximately 10 grams may be fatal in an adult (Akintonwa *et al*., 1989). The main brunt of copper sulfate is borne in the order by erythrocytes liver and then kidney leading to methanoglobinemia, hepatotoxicity and renal failure (Blundell *et al*., 2003). Uptake of copper from the intestines is susceptible to competitive inhibition by other transition metals (particularly zinc or iron). The presence of dietary proteins and amino acids,

complexion or precipitating anions, fructose, ascorbic acid, folic acid and fiber may also influence copper uptake from the gastrointestinal tract (Institute of Medicine, 2001). Copper increase, zinc decrease, this correlation seems to be caused by the competitive absorption relationship of zinc and copper within enterocytes, mediated by metallothionein MT, expression of MT is up regulated by high dietary zinc content, MT binds copper with a higher affinity than zinc (Ogiso, 1979 and Igic, 2002). Zinc induced copper deficiency leading to anemia were reviewed by Fiske and colleagues (Fiske *et al*., 1994). One of the most common trace-metal imbalances is elevated copper and depressed zinc (the optimal plasma or serum ratio is 0.70 - 1.00) the ratio of copper to zinc is clinically more important than the concentration of either of these trace metals, Copper and zinc are regarded as neurotransmitters and are in high concentrations in brain hippocampus (Fischer *et al*., 1981). Elevated copper and depressed zinc have been associated with hyperactivity, attention deficit disorders, depression. Also paranoid schizophrenia, in addition to other biochemical imbalances (Malarveni *et al*., 2010). Zinc is an essential trace element that is crucial to survival and health maintenance, as well as growth, development, and maturation organisms of all animal species (Harlal, 2005). Taking high levels of zinc supplements over long periods of time may lower the body's ability to absorb copper (Steven *et al*., 2011). On the other hand the heavy metals that were previously stored in safer hiding places this process is called retoxification, it can easily be avoided by simultaneously giving an intestinal toxin-absorbing agent. A recent animal study demonstrated rapid removal heavy metals from the brain and skeleton superior to any known other detox agent (Joseph, 2001 and Dietrich, 2007). *Coriandrum sativum* or Cilantro is a common Mexican and Middle Eastern spice (Williams, 1998) which has been found to chelate or removal heavy metals (mercury, lead, copper, etc.), it is believed to cross the blood-brain barrier and actually remove said metals from the brain (Williams, 1998).

Material and Methods

The chronic toxicity study involves 110 male mice which were divided in to 11 groups each group contains 10 animals as following:

Group (1): treated orally by stomach tube with distilled water this group considered as control negative (1).

Group (2): treated orally by stomach tube with 8 mg/ kg.bw $CuSO_4$ for 8 weeks and then treated orally with distilled water this group considered as control positive (1).

Group (3): gavages by stomach tube 40 mg/kg.bw $CuSO_4$ for 8 weeks then treated orally with distilled water this group considered as control positive (2).

Group (4): gavages by stomach tube 8 mg/kg.bw $CuSO_4$ + 70 mg/kg.bw zinc oxide for 8 weeks.

Group (5): gavages by stomach tube 40 mg/kg.bw of CuSO4 followed by 70 mg/kg.bwof zinc oxide for 8 weeks.

Group (6): gavages by stomach tube with 8 mg/kg.bw of $CuSO_4$ followed by 50 mg/kg.bw of Cilantro and 35mg\kg.bw of zinc oxide for 8 week.

Group (7): gavages by stomach tube with 40 mg/kg.bw of $CuSO_4$ followed by 50mg/kg.bw of Cilantro and 35mg\kg.bw of zinc oxide for 8 week.

Group (8): gavages by stomach tube with 8 mg/kg.bw of $CuSO_4$ fallowed by 100 mg/kg.bw of Cilantro for 8 weeks.

Group (9): gavages by stomach tube with 40 mg/kg.bw of $CuSO_4$ fallowed by 100 mg/kg.bw of Cilantro for 8 weeks.

Group (10): gavages by stomach tube with 70 mg/kg.bw of zinc oxide for 8 weeks.

Group (11): gavages by stomach tube with 100 mg/kg.bw of Cilantro for 8 weeks.

Result and Discussion

At the end of the each period of experiment, three animals from each group were sacrificed by chloroform and post mortem examination was done for all animals. The macroscopic appearance was recorded to detect any abnormal gross changes in internal organs. Specimens were taken from all targeted organs; the tissues were kept in 10% formaldehyde solution, for fixation, and then processed routinely by using the histokinate. Tissue sections were embedded in paraffin blocks, and sectioned by microtome with hematoxylin and eosin, then examined by using light microscope (Luna and Lee, 1968).

Liver: The hepatic parenchyma showed enlargement of hepatocytes with large nuclei showing chromatolysis, the nuclei contain more than prominent nucleoli, with diffuse minimal vacuolation and tiny aggregation of neutrophils in the parenchyma (Figure-1).The liver showed enlargement of hepatocytes due to diffuse vacuolation in the parenchyma of liver agreed with (Attia *et al*., 2009).

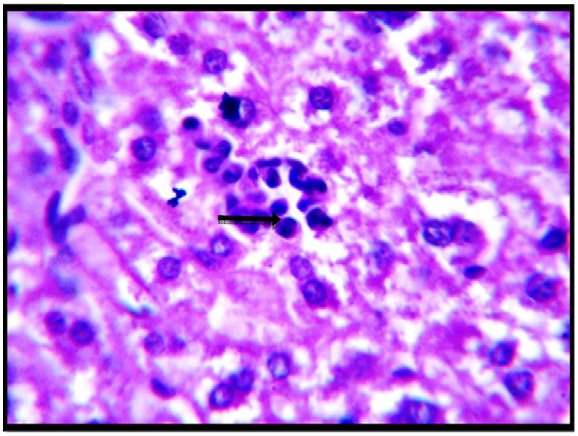

Fig. 1 : Liver of mouse treated with (8 mg/kg. bw. $CuSO_4$) after 20 days of toxicity showing tiny aggregation of neutrophils in the parenchyma (arrow) (H&E 400X).

Kidneys: The main lesions were restricted to the cortical area, characterized by degeneration and necrosis the of the epithelial lining of the proximal and distal convoluted tubules with formation of renal cast, the glomeruli showed atrophy of glomerular tuft with dilation of Bowman's space (Figure-2).These changes are more severe in high dosage group and that may be attributed to the intravascular hemolysis and releasing Cu that's may be lead to necrosis of the epithelial lining of the tubules this result was in agreement with (Chugh, 1977 and Dashy, 1989).

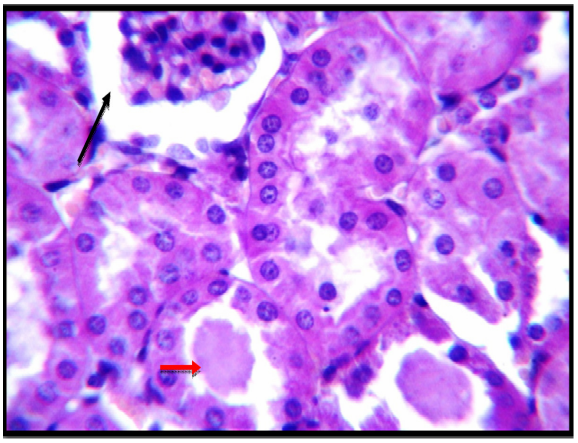

Fig. 2 : Kidney of mouse treated with (8 mg/kg bw. $CuSO_4$) after 20 days of toxicity showing renal cast (→) and atrophy of glomerular tufts (→) (H&E 400X).

Spleen: Severe congestion of blood sinuses with proliferation of megakaryocytic. Large numbers of megakaryocytic in the red pulp region that extra medullary hemopioesis take place in the spleen as a secondary mechanism that's agreed with (Jones and Hunt, 1983). Brain: Perivascular and perineuronal edema and congestion of the cerebrum, the meninges showed severe hemorrhage and infiltration of neutrophils. Perivascular and perineuronal edema which is clear in all section. The amount of the fluid increased with the time of experiment especially in high dose, with extensive demyelination of white matter with shrinkage of neurons contains pyknotic nuclei, there are extensive areas of necrosis and hemorrhage in the cerebrum with infiltration of neutrophils that's agree with (Al-Naimi *et al*., 2010). Congestion of cerebral and meningeal blood vessels was also seen. The cerebellum showed edema between the molecular and granular with degeneration of some purkingi cell and complete dissolution of the others (Figure-3). The main lesions at this period was the clustering of astrocytes around of necrosis areas, these lesions could be attributed to the toxic effect of $CuSO_4$. It Caused cell injury by damage of lysosome membrane leading to release of Cu in cytoplasm of cell as explained by (Lirquist, 1968).

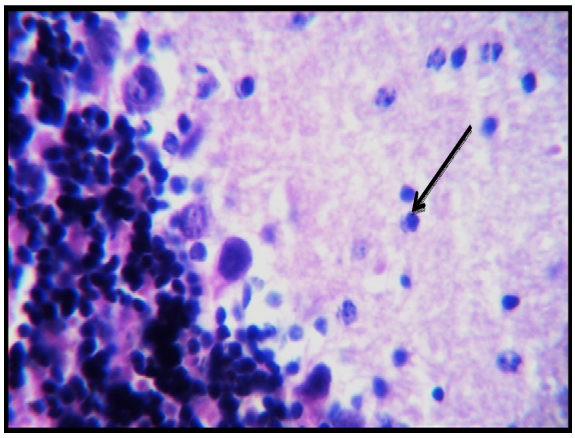

Fig. 3 : Brain of mouse treated with (8 mg/kg bw. $CuSO_4$) after 20 days of toxicity showing degeneration of purkingy cells (←) (H&E 400X).

Stomach: The non-glandular region showed marked hyperkeratosis and papillary hyperplasia of the epithelial lining of the mucosa (Figure-4). While the glandular region showed infiltration of neutrophils and mononuclear cells in and between the sub mucosal glands. The study showed marked hyperkeratosis and papillary hyperplasia of the epithelial lining of the mucosa with marked change were seen in high doses and development of experimental period which accrue due to toxic effect of $CuSO_4$ (Magdalena *et al*., 2004).

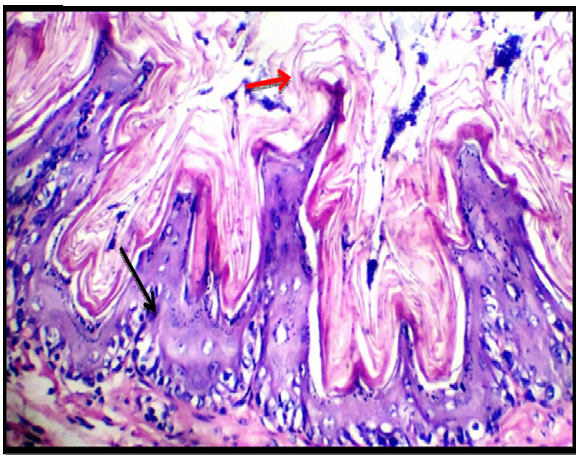

Fig. 4 : Stomach (non-glandular region) of mouse treated with (8mg/kg bw. $CuSO_4$) after 20 days of toxicity showing marked hyperkeratosis (→) and papillary hyperplasia of the epithelial lining of mucosa (→) (H&E 400X).

Lung: Thickening of interalveolar septa due to congestion of alveolar blood capillaries and infiltration of inflammatory cells mainly neutrophils (Figure-5). Thickening of interalveolar septa due to congestion of alveolar blood capillaries and infiltration of inflammatory cells mainly neutrophils forming broncho-pneumonia, the epithelial cells lining the bronchioles showed papillary hyperplasia with increase in the number of goblet cells that lead to severe excretion for mucin with infiltration for inflammatory cells lead to suppurativebroncho-pneumonia that's not agree with (Hantson *et al*., 1996).

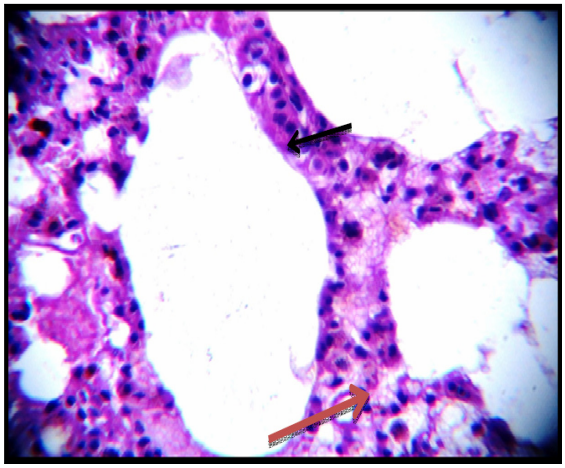

Fig. 5 : Lung of mouse treated with (8mg/kg bw. $CuSO_4$) after 20 days of toxicity showing thickening of the interalveolar septa (→) due to congestion of alveolar blood capillary and inflammatory cells infiltration (→) (H&E 400X).

Adrenal Gland: suffered from vacuolation of zonagranulosa. Some sections show elongation of zona fasciculate and zonareticularis with decrease in the size of medulla vacoulation of zonagranulosa. This occur at large dose and long period of treatment. These changes occur due to toxic effect of $CuSO_4$ on hypothalamic-pituitary adrenal axis function which would be effects on secretion and metabolism of adrenocortical hormones that's agreed with (Bousquet-Moore *et al*., 2008). (Figure-6).

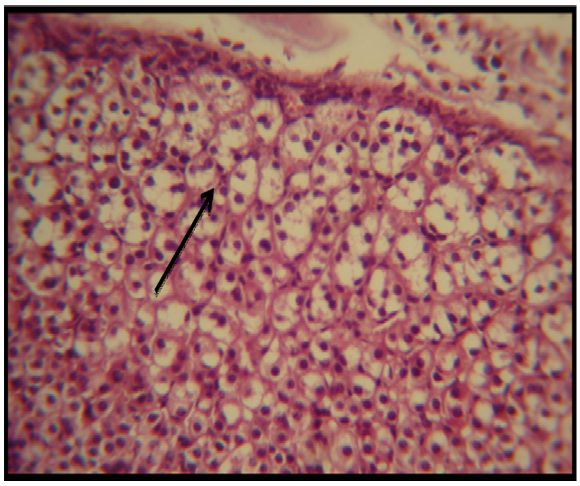

Fig. 6 : Adrenal of mouse treated with (8mg/kg bw. $CuSO_4$) after 20 days of toxicity showing vacuolation of zonagranulosa (→) (H&E 100X).

Liver: The histopathological changes showed similar picture as in the previous group, in addition there is fatty degeneration and mononuclear cells infiltrated in the periportal area with individualization of hepatocytes and increase in the rate of apoptosis. That's agreed with (Attia *et al*., 2009) due to diffuse minimal vacuolation in the parenchyma of liver. **Kidneys**: Similar lesions as in the previous group furthermore there is degeneration and necrosis of epithelial lining of cortical renal tubules, with severe interstitial hemorrhage.These changes are more severe in high dosage group and that may be attributed to the intravascular hemolysis and releasing Cu that's may be lead to necrosis of the epithelial lining of the tubules this result agree with (Chugh, 1977 and Dashy, 1989). **Intestine**: Increase in the number of goblet cells with accumulation of large amount of mucous in the lumen. **Heart**: There is edematous fluid between muscle fiber. Many myocardial cells showed minimal vacuolation with congestion of blood vessels. Pancreas: Tissue sections showed atrophy of islant of langerhans. **Liver**: The main histopathological lesion at this period was the formation of hyperplastic nodules, these nodules consisting of large hepatocytes with no arrangement lacking the central vein and cause pressure atrophy to the adjacent liver parenchyma (Figure-7). Continuously of treatment by $CuSO_4$ showing

hyperplastic nodules this change accure due to copper accumulation and liver is maintaining its ability of regeneration and produced new hepatocyte (Kato *et al.*, 1996).

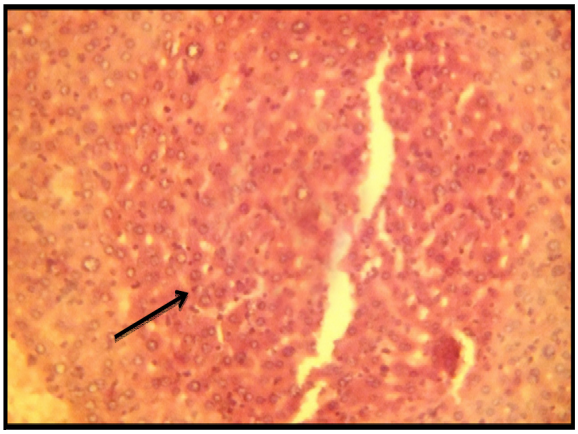

Fig. 7 : Liver of mouse treated with (8mg/kg.bw. $CuSO_4$) after 40 days of toxicity showing hyperplastic nodules causing pressure atrophy to the liver parenchyma (→) (H&E100X).

Kidneys: The degeneration and necrosis of epithelial lining of the cortical renal tubules are more severe than the previous period with deposition of hemosiderin pigment (Figure-8).

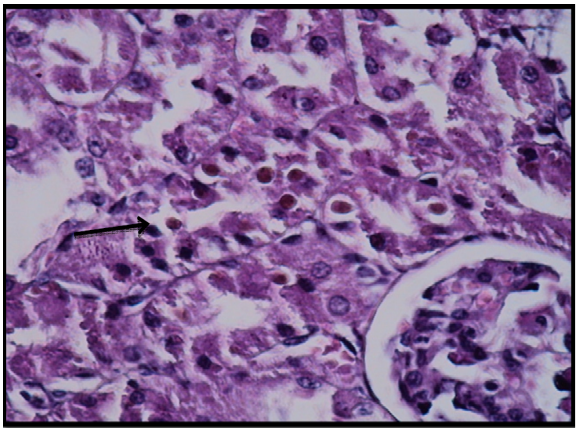

Fig. 8 : Kidney of mouse treated with (8 mg/kg. bw. $CuSO_4$) after 40 days of toxicity showing deposition of hemosiderin pigment (→) (H&E400X).

Brain : Increase in amount of edema than the previous period.

Spleen: Showed similar histopathological change as in the previous period. Depositions of hemosiderin pigment within the erythrocytes were also seen (Figure-9). Deposition of hemosiderin pigment and in high dose at same period the congestion was more severe and large amount of hemosiderin pigment deposition that's agree with (Anderson, 1989; Susan, 2002 and Irene 2002). Other organs showed the same lesions as in the previous period.

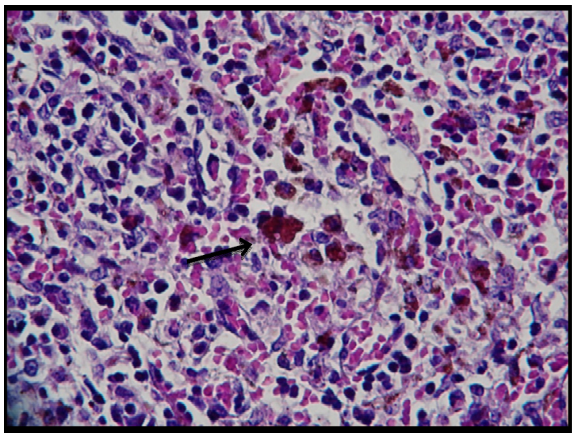

Fig. 9 : Spleen of mouse treated with (8mg/kg bw. $CuSO_4$) after 40 days of toxicity showing deposition of hemosiderin pigment (→) (H&E.

Liver : Tissue sections showed similar lesions as in the group treated with 8mg/kg of the same period in addition to increase in neutrophils infiltration especially in the portal areas which showed mononuclear cells infiltrate and slight fibrosis .Slight fibrosis of the central veins wall were also seen.

Spleen: The congestion becomes more severe than the previous period with increase in amount of hemosiderin pigment. Slightly fibrosis of the capsule was also noticed.

Heart: Extensive edema of the myocardium leading to displacement of muscles fibers in addition to severe dilatation and congestion of myocardial blood vessels.

Brain: Demyelination of white matter with shrinkage of neurons contains pyknotic nuclei (Figure-10).With shrinkage of neurons contains pyknotic nuclei, there are extensive areas of necrosis and hemorrhage in the cerebrum with infiltration of neutrophils.

The main lesions at this period was the clustering of astrocytes around of necrosis areas, these lesions could be attributed to the toxic effect of $CuSO_4$. It Caused cell injury by damage of lysosome membrane leading to release of Cu in cytoplasm of cell as explained by (Lirquist, 1968).

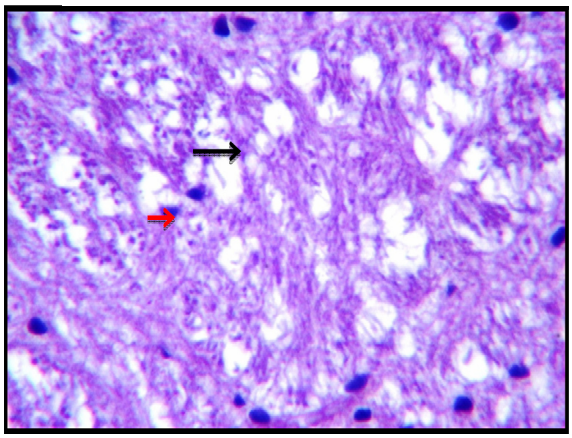

Fig. 10 : Brain of mouse treated with (40mg/kg bw. $CuSO_4$) after 40 days of toxicity showing demyelination of white matter (→) with neurons containing pyknotic nuclei (→) (H&E400X).

Lung: Showed chronic suppurativebroncho-pneumonia characterized by infiltration of large numbers of neutrophils within the alveolar, bronchial and bronchiolar Lumina. The epithelial cells lining the bronchioles showed papillary hyperplasia with increase in the number of goblet cells. Infiltration of inflammatory cells mainly neutrophils forming broncho-pneumonia, the epithelial cells lining the bronchioles showed papillary hyperplasia with increase in the number of goblet cells that lead to severe excretion for mucin with infiltration for inflammatory cells lead to suppurativebroncho-pneumonia that's not agree with (Hantson *et al*., 1996).

Liver: There are wide areas of parenchymal necrosis, blood oozing to the necrotic areas (Figure-10).

Kidneys: In addition to the previous lesions .There is severe mononuclear cells infiltration in the interstitial tissue causing pressure atrophy to the renal tubules. The pelvic area showed infiltration of neutrophils. The pelvic area of kidney showed in this study present infiltration of neutrophils in high dose with long period of intoxication there is slight interstitial and periglomerular fibrosis causing thickening of Bowman's capsule and effect on the renal parenchyma these result agree with (Nurullah *et al*., 2006).

Spleen: The congestion become more severe with deposition of large amount of macrophages laden the hemosiderin pigment.

Heart: The myocardium showed fatty degeneration with infiltration of mononuclear cells and congestion of blood vessels. There is edematous fluid between muscle fiber. Many myocardial cells showed minimal vacoulation with congestion of blood vessels. With increasing dose of $CuSO_4$ and continuous the time of experiment an extensive edema of the myocardium leading to displacement of muscles fibers, the myocardium showed fatty degeneration and congestion of blood vessels with hemorrhagic and necrosis with infiltration of mononuclear cells and fibrosis that's lead to developed of necrosis this agree with (Salonen *et al*., 1991), they state there was positive correlation between serum Cu levels and risk of acute myocardial infarction.

Pancreas: Severe hemorrhage with infiltration of neutrophils section showed Severe hemorrhage with infiltration of neutrophils and marked fibrosis leading to atrophy and absence of islands of Langerhans with infiltration of mononuclear cells that's agree with (Al-Tayar, 2008).

Kidney: In addition to previous lesion seen at the 40 day of intoxication there is slight interstitial and periglomerular fibrosis causing thickening of Bowman's space (Figure-11). The pelvic area of kidney showed in this study present infiltration of neutrophils in high dose with long period of intoxication there is slight interstitial and periglomerular fibrosis causing thickening of Bowman's space and effect on the renal parenchymia. These result agree with (Nurullah *et al*., 2006). They report that chronic tubular interstitial nephritis had developed as a consequences of parenteral $CuSO_4$ in human.

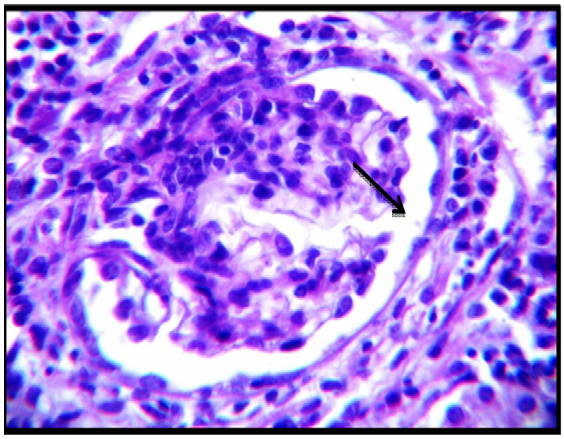

Fig. 11 : Kidney of mouse treated with (40mg/kg. bw. of $CuSO_4$) after 60 day of toxicity showing slight priglomerular fibrosis leading to thickening of Bowman's capsule (→) (H&E 400X).

Liver: Periportal fibrosis with infiltration of inflammatory cells mainly mononuclear cells in addition to hyperplasia of bile ductules characterized by epithelial lining cells contains hyper chromatic nuclei. **Brain**: The main lesions at this period were the clustering of astrocytes around of necrosis areas, blood vessels showed necrosis of smooth muscles cells of the tunica media and infiltration which is fallow by cellular

accumulation in the tunica adventitia companied by swelling of endothelial cells, perivascular and perineuronal edema which was clear in all section. The amount of the fluid increased with the time of experiment especially in high dose, with extensive demyelination of white matter that's agree with [30]. **Heart**: There is severe damage of myocardial cells with moderate fibrosis. **Pancreas:** Marked fibrosis leading to atrophy and absence of islants of langerhans with infiltration of mononuclear cells. The pancreatic degenerative changes may lead to pancreatic insufficiency of both the exocrine and endocrine system consequently impacting on the liver lesions, for example, the conversion of glycogen to glucose, which occurs in the liver and is stimulated by glucagon from the endocrine pancreas, is likely to be affected which is one of causative agents for vacoulation in the liver that's agreed with (Irene, 2002). **Intestine**: In addition to the previous lesions there is mononuclear cells infiltration in the lamina propria of mucosa. **Skin**: Beside hyperkeratosis and acanthosis there is increase in sclerosis of the dermis leading to absence of dermal appendage (Figure-12). Examined sections showed acanthosis and hyperkeratosis and theselesions increased with large dose and continuously with experiment time forming focal hypertrophyof the skin in addition to hyperkeratosis and acanthosis there is increase in collagen "sclerosis" with cystic dilation of hair follicles and alopecia which characterized by reduction in number of hair follicles. These agreed with (Al-Naimi *et al*., 2010).

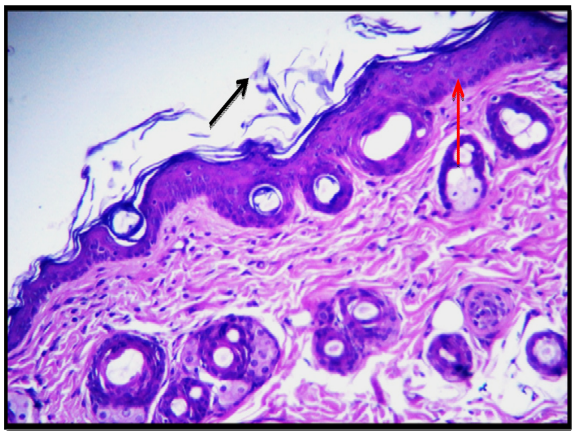

Fig. 12 : Skin of mouse treated with (40mg/kg. bw. of $CuSO_4$) after 60 day of toxicity showing hyperkeratosis (→) and acanthosis (→) there is increase in sclerosis of the dermis (H&E 200X).

Kidneys: Perivascular lymphocytic cuffing of intertubular blood vessels in all doses seen at the first period of treatment especially at doses 40 mg/kg bw. of $CuSO_4$. In addition to focal interstitial aggregation of mononuclear cells. In tissue section of kidney showed there is perivascular lymphocytic cuffing of intertubular blood vessels and in spleen tissue sections showed lymphoid hyperplasia of white pulp and during long period of cilantro treatment the organs showed deposition of amyloid like substance. The changes in tissue section treated by $CuSO_4$ and treated by Cilantro explain the role of cilantro as anti-oxidant and as Immunostimulator that's agreed completely with (Neamah *et al*., 2012). **Spleen**: Tissue sections showed lymphoid hyperplasia of white pulp in treated group of toxic doses at 20 and 40 days of treatment while at 60 days period the organ showed the deposition of amyloid like substance (Figure-13).

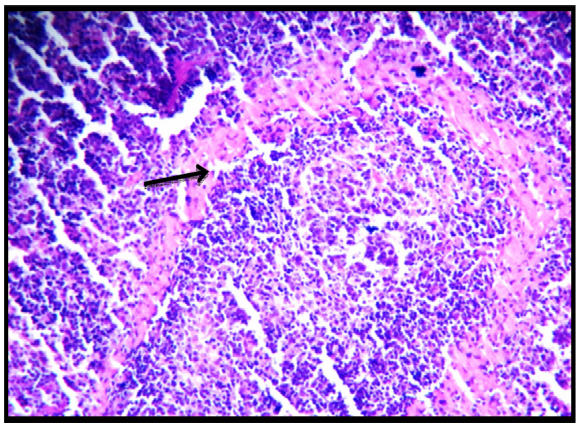

Fig. 13 : Spleen of mouse treated with Cilantro showing focal deposition of amyloid like substance (→) (H&E20X).

Lung: All treated group showed perivascular and peribronchiolar lymphocytic cuffing especially at dose 8 mg/kg. bw. of $CuSO_4$ treatment. Treated by zinc showed perivascular and peribronchiolar lymphocytic cuffing in tissue section of lung this change appear after treated by Zn as a results of Zn effect against $CuSO_4$ toxicity. **Liver**: Early granuloma in the hepatic parenchyma consisting of neutrophils and epithelioid cells at 40 days of treatment (Figure-14). The granuloma appeared beside the congested blood vessels at 60 days of treatment. Hepatic sections in group of treatment by Zn showed kupffer cells activation in all supplemented group along the period of experiment that's agreed with (Shengying *et al*., 2010). Formation of early granuloma in the hepatic parenchyma, in kidney the treated by combination the cortical regions showed the formation of new renal tubules characterized by epithelial lining cells contain hyper chromatic nuclei, in brain tissue section showed Perivascular cuffing of cerebral blood vessels.

That does agree with (Debjit *et al*., 2010). They found that zinc supplement is used to commonly to enhance wound healing.

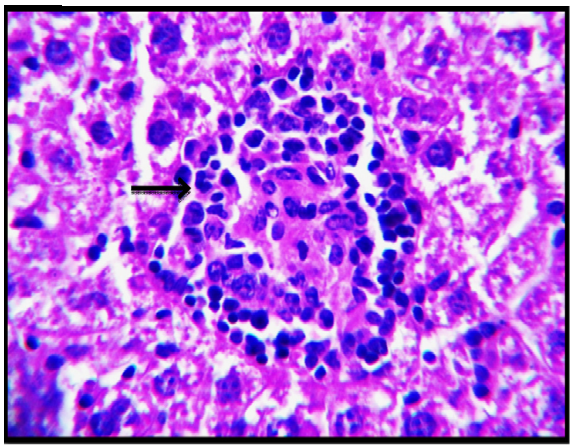

Fig. 14 : Liver of mouse treated with Zinc showing formation of early granuloma in the hepatic parenchyma (→) (H&E 400X).

Heart: The myocardium showed the formation of granuloma between the myocardial especially at later stage of treatment. **Intestine:** Extensive proliferation of lymphoid tissue of sub mucosa with formation of germinal center. **Brain:** Perivascular cuffing of cerebral blood vessels (Figure-15)

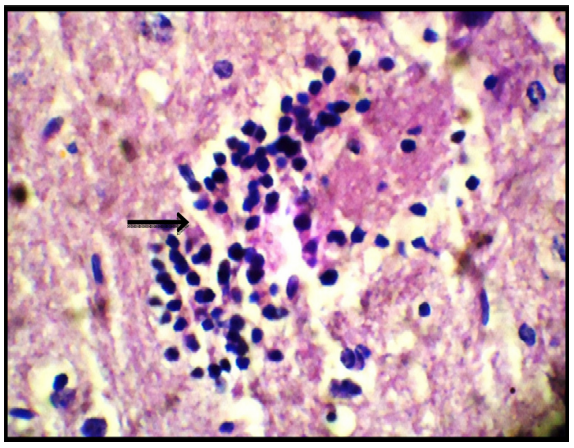

Fig. 15 : Brain of mouse treated with combination of Zinc and Cilantro at 40 days period showing focal gliosis (→) (H&E 400X).

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