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Histopathology and residues in fresh water fish exposed to acute and chronic copper and mercury toxicity

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A total number of 668 apparently healthy fish were obtained from farm to study the effect of two heavy metals (Copper and Mercury) on histopathology of liver, kidney, spleen, gills and muscles also residues in muscles. The LC_{50} /96 hr. of Cu and Hg were estimated and fish exposed to ½ LC_{50} for 7 days and for $1/_{10}$ LC_{50} for 8 weeks from each product separately. Histopathological findings in acute and chronic mercuric chloride toxicity revealed degeneration and necrosis in the glomeruli, interstitium tissue and epithelium lining renal tubules. The tubular epithelium became necrotic at several places. Eosinophilic hyaline droplets is exist in the cytoplasm of the necrosed cells. Degenerative changes and hyperactivity in melanomachrophage center was seen in the spleen together with some necrotic areas. Necrosis and aggregation of melanomachrophage were seen in the hepatic cells, Hepatic cells showed vacuolar degeneration in the hepatic cells. Gills showed loss in the lamellae of the filaments associated with edema, inflammatory cells infiltration and haemorrhages in the arch. The sarcoplasm of the bundles of the skeletal muscle showed granular degeneration and focal inflammatory cells infiltration between the hyalinized bundles. Mercury residues obtained from these studies in the acute toxicity were 0.22 ppm/gm in the 2nd day, 0.411 ppm/gm in the 5th day ended with 0.96 ppm/gm in the 7th day. In chronic toxicity it was 1.1320, 1.7140, 2.3620 and 3.5640 ppm/gm respectively from the 2nd to the 8th week of exposure. In acute and chronic copper toxicity, there was degenerative changes in renal tubules. Melanophores aggregation in the wall of the blood vessels of the spleen and depletion of some of the melanophores in the melanomachrophage were seen together with necrosis in some areas. Congested Mvs (Micro vessels) and vacuolation of hepatocytes were observed. Some areas of hemorrhage and melanophores vacuolar degeneration in the liver were seen. There was mitosis in some areas with displesia of hepatopancreatic cells and eosinophilic granular cells aggregation. Zymogen granules disappeared and there were dyplastic hepatocytes. Congestion in the blood vessels of the gill filaments, associated with massive number of granular eosinophilic cells infiltration were seen in the base of the filaments. There were sever vacuolization and hyalinization in the skeletal muscle bundles. Detection of residues of copper sulfate revealed increase of the amount of copper measured in ppm/gm comparing to the normal control starting from 0.60 ppm/g in the 2nd day, 0.67 ppm/g in the 5th day and 0.67 ppm/g in the 7th day. Result obtained in chronic copper sulfate toxicity revealed gradual increase of the amount of copper which ranged from 0.18 ppm/g at the 2nd week to 0.21 ppm/g in the 8th week of exposure.

Key words: Histopathology, *Tilapia nilotica*, Copper and mercury toxicity, Residues

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Pollution of lake, river and sea water by trace heavy metals occurs from human industrialization activities that include emissions from automobiles, coal burning mining, industrial activities and trash incineration, besides the discharge of domestic and industrial wastewaters and the dumping of sewage sludge. This pollution may lead to an increase of trace heavy metals level in aquatic habitats and the sediments. (Hakanson, 1984; Dethloff et al., 1998 and Shotyk et al., 1998).

Heavy metals, such as cadmium, copper, lead, chromium and mercury are important environmental pollutants particularly in areas with high anthropogenic pressure. Their presence in the atmosphere, soil and water, even in traces, can cause serious problems to all organisms, and their bioaccumulation in the food chain can be highly dangerous to human health (Ejaz et al., 2007 and Glenn et al., 2009). Nowadays human beings are extremely scared from consumption of animal and bird protein because of the presence of outbreaks of some epidemic diseases such as bird flu, swine flu and bovine spongio- encephalopathy (BSE). Therefore, fish is an important source of protein that can substitute animal and poultry protein. Contamination of fresh water streams with a wide range of pollutants has become a matter of concern over the last few decades (Dirilgen 2001 and Vutukuru 2005).

Materials and Methods

Six hundred and sixty eight apparently healthy Tilapia nilotica fish (Oreochromis niloticus) of both sexes, weighing 130-170 g. and of 20-25cm length were used. They were gathered from El-Wafa farm, Manyal Sheha, Giza Province. During their transportation to the laboratory, fish were kept in large plastic containers filled with the same water source and spilled with air by portable aerator.

Each of mercury (Hg Cl_2, MW 271.50 provided by Rhone-Poulenc-Paris, France) and copper sulfate ($CuSo_4$; MW 249.68 provided by El-Nasr-Adwic Company-Egypt) were added to aquarium's water in different concentrations of LC_{50}/ 96 hr during short term and long term exposure experiments.

Fish were scarified and tissue specimens from kidneys, liver, spleen, gills and muscles were tested according to Jules (1982). Samples from muscles were prepared for residual analysis according to (Koirtyohann et al., 1982).

Results

Short term exposure to sublethal dose ($\frac{1}{2}$ LC_{50}/ 96 hr.) of mercuric chloride in Tilapia nilotica

Postmortem findings of Tilapia nilotica exposed to ½ LC_{50}/ 96hr. of mercuric chloride showed congestion of kidney, spleen, liver and intestine. The gall bladder was distended and the stomach appeared congested and dry along the period of the experiment.

Histopathological findings 2nd day post exposure declare in sections from the kidneys a hemorrhage and necrosis in melano-machrophage center (Fig. 1a) and destruction in the epithelial lining of the collecting ducts (Fig. 1b).Sections from spleen showed hemorrhagic areas (Fig. 1c).No histopathological changes were observed in liver. Vacuolization and hyalinization with eosinophilic cell infiltration was observed in the bundles of the skeletal muscles (Fig. 1d). Gills showed eosinophilic cells infiltration in the base of the filaments (Fig. 1e) associated with losses of the lamellae (Fig. 1f). In 5th day kidney sections revealed eosinophilic hyaline droplets in renal tubules (Fig. 2a). Splenic changes were similar to those detected on the 2nd day. Liver sections showed congestion and degenerative changes in hepatocytes (Fig. 2b). There were hyalinization and swelling in the bundles of the skeletal muscles (Fig. 2c). The gills showed loss in the lamellae of the filaments and were infiltrated by eosinophilic cells at the base (Fig. 2d). The arch showed hemorrhagic areas (Fig. 2e).

In 7th day Kidney sections showed a hypercellularity of glomerular capillary tufts and necrosis in re-

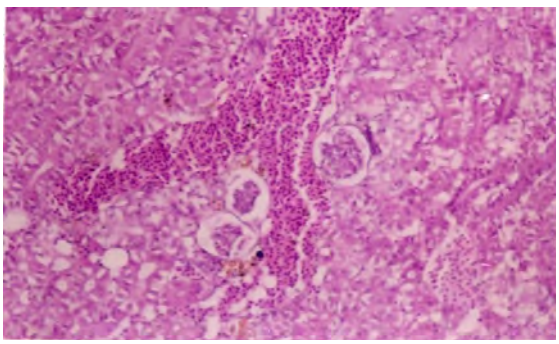

Fig. (1a): Kidney 2 days after exposure to mercuric chloride showing Hemorrhage and necrosis in melano-machrophage center.
H&E (X200)

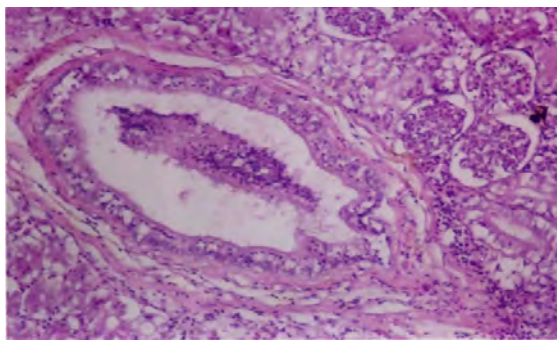

Fig. (1b): Kidney 2 days exposure to mercuric chloride showing destruction in the epithelial lining the collecting ducts.
H&E (X200)

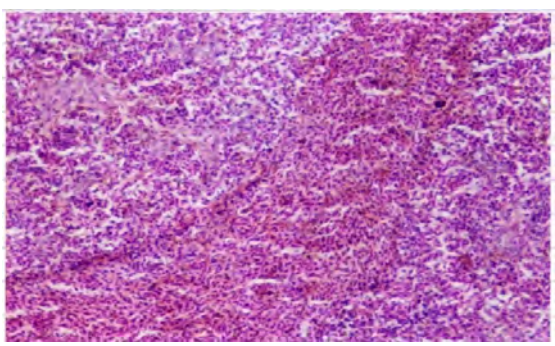

Fig. (1c): Spleen 2 days after exposure to mercuric chloride notice area of hemorrhage.
H&E (X200)

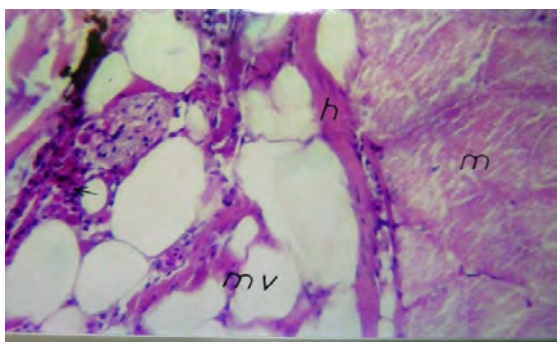

Fig. (1d): Skeletal muscles 2 days after exposure to mercuric chloride notice vacuolization (mv) and hyalinization (h) with eosinophilic cells infiltration in the bundles. (arrow).
H&E (X80)

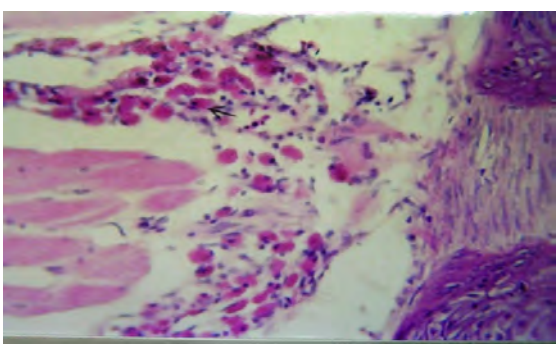

Fig. (1e): Gills 2 days after exposure to mercuric chloride showing eosinophilic cells infiltration in the base of the filaments. (arrow)
H&E (X80)

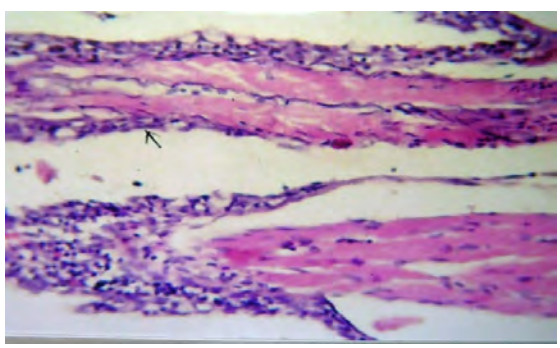

Fig. (1f): Gills 2 days after exposure to mercuric chloride showing loss of the lamellae of the filaments. (arrow)
H&E (X80)

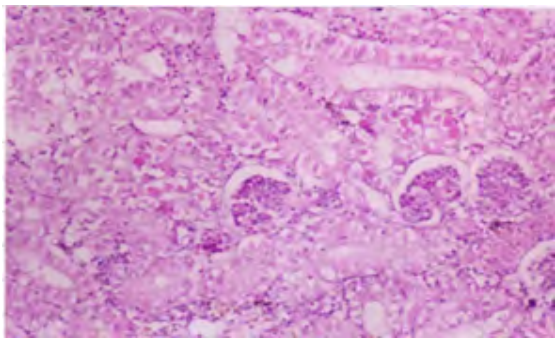

Fig. (2a): Kidney 5 days after exposure to mercuric chloride notice eosinophilic hyaline droplets in renal tubules.
H&E (X200)

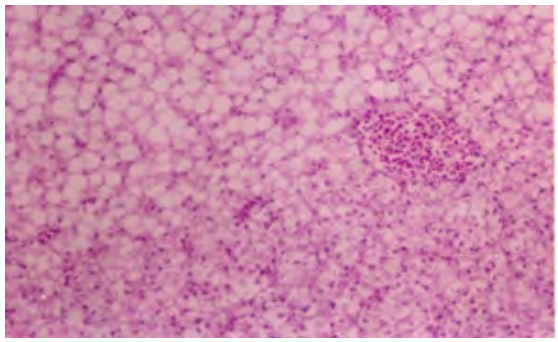

Fig. (2b): Liver 5 days after exposure to mercuric chloride showing congestion and degenerative changes in hepatocytes.
H&E (X200)

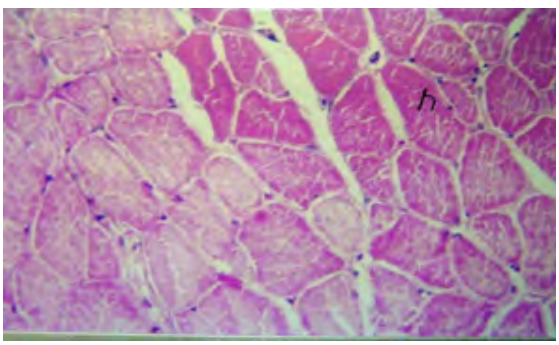

Fig. (2c): Skeletal muscles 5 days after exposure to mercuric chloride showing hyalinization (h) and swelling in the bundles.
H&E (X80)

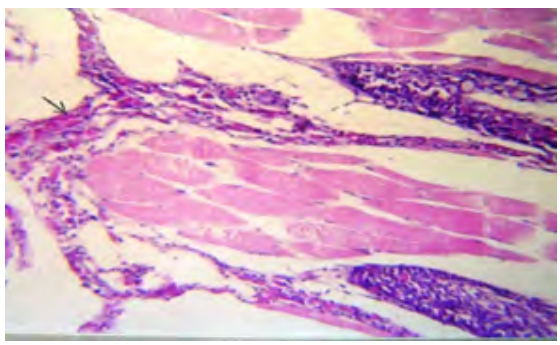

Fig. (2d): Gills 5 days after exposure to mercuric chloride notice Infiltration by eosinophilic cells at the base of the filament. (arrow)
H&E (X64)

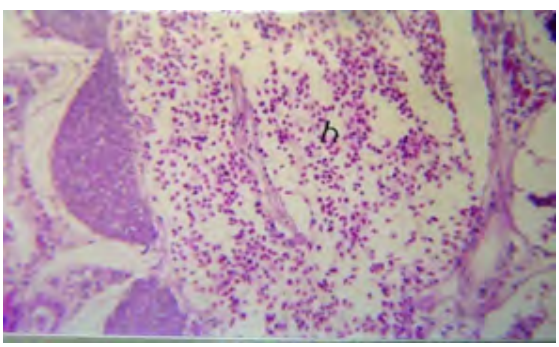

Fig. (2e): Gills 5 days after exposure to mercuric chloride notice hemorrhagic areas in the gill arch (h).
H&E (X40)

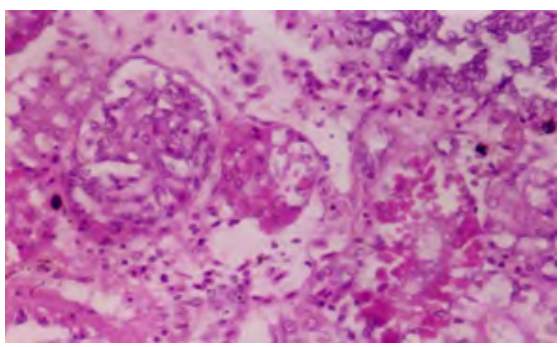

Fig. (3a): Kidney 7 days after exposure to mercuric chloride showing. hypercellularity of glomerular capillary tufts and necrosis in renal tubules.
H&E (X400)

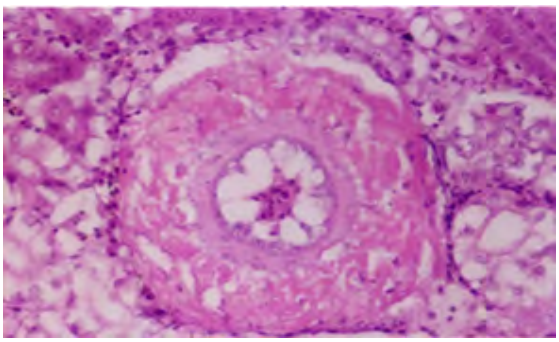

Fig. (3b): Kidney 7 days after exposure to mercuric chloride manifest the swelling of endothelium and thickening of blood vessels wall.
H&E (X800)

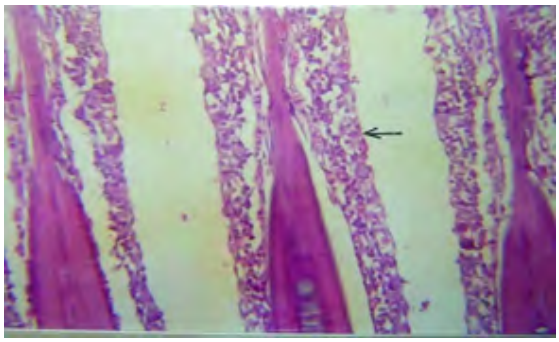

Fig. (3c): Section in gills 7 days after exposure to mercuric chloride showing loss of the lamellae. (arrow)
H&E (X40)

nal tubules (Fig. 3a) together with swelling of endothelium and thickening of blood vessels wall (Fig. 3b). Changes of the liver and spleen were similar to those observed in the 5th day. The bundles of skeletal muscles showed vacuolization and hyalinization while the gills showed eosinophilic cells infiltration in the base of the filaments associated with loss of the lamellae (Fig. 3c).

Detection of residues in Tilapia nilotica muscles exposed to ½ LC_{50} /96-hr for 7 days of mercuric chloride revealed an increase in the amount of residue (ppm/gm) in comparison to the control. Values were 0.22, 0.41 and 0.96 ppm/gm at the 2nd, 5th and 7th days respectively (Table 1).

Table 1. Residues in fish muscles (ppm/g) after exposure to mercuric chloride & copper sulfate ½ LC_{50}/ 96 hr for 7 days

Time (days)	Group	Mercuric chloride Measurement (ppm/g)	Copper sulfate ppm/gm muscle
2	C	0.00	0.11
	T	0.22	0.60
5	C	0.11	0.12
	T	0.41	0.67
7	C	0.26	0.14
	T	0.96	0.67

C = Control, T = Treated

Long-term exposure to sublethal dose ($^1/_{10}$ LC_{50}/ 96 hr.) of mercuric chloride in Tilapia nilotica fish

Postmortem findings of Tilapia nilotica fish exposed to $^1/_{10}$ LC_{50}/ 96 hr. of mercuric chloride showed friable and congested parts of the kidney, yellowish pale coloration of the liver with greenish border and enlargement of the gall bladder.

In 2nd week liver revealed an eosinophilic granules and necrosis of hepatic cells while kidney showed vacuolar degeneration in the renal tubules and degenerative changes in the melanomachrophage center. Necrotic changes in the spleen were also noticed. Few inflammatory cells infiltration was observed in between the bundles of skeletal muscles (Fig. 4a), associated with focal pigmentation in the perivascular area between the bundles (Fig. 4b). Gills showed congestion in the filaments (Fig. 4c); associated with oedema, eosinophilic cells and leucocytes inflammatory cells infiltration in the arch (Fig. 4d). In 4th week liver showed an area of necrosis and eosinophilic granules (Fig. 5a). Some necrotic areas were seen in the kidney same as in the 2nd week. Perivascular pigmentation was observed in between the bundles of skeletal muscles (Fig. 5b), associated with hyalinization. Gills showed loss of the lamellae of the filaments (Fig. 5c) associated with oedema, inflammatory cells infiltra-

Table 2. Residues in fish muscles (ppm/g) exposed to copper sulfate & mercuric chloride 1/10 LC_{50}/96 hr. for 8 weeks

Time (weeks)	Group	Mercuric chloride ppm/gm muscle	Copper sulfate ppm/gm muscle
2	C	0.3820	0.12
	T	1.1320	0.18
4	C	0.5040	0.09
	T	1.7140	0.18
6	C	0.8670	0.10
	T	2.3620	0.19
8	C	1.0360	0.12
	T	3.5640	0.21

C = Control, T = Treated

tion and hemorrhages in the arch. In the 6th week of exposure spleen revealed hyperactivity of the melano-machrophage center. Glomelular atrophy and necrosis in the kidney was seen. Liver showed perivascular aggregation of the melanomachrophages center and degenerative changes in the hepatic cells. The sarcoplasm of the bundles of the skeletal muscles showed granular degeneration (Fig. 6a). Inflammatory cells infiltration was observed in the base of the gill filaments, while the arch showed also inflammatory cells infiltration with dilatation in the blood vessels (Fig. 6b).

In 8th week spleen revealed similar lesions to that appeared in the 6th week (Fig. 7a) in addition to glomerular atrophy, necrosis (Fig. 7b) and melanophores in the epithelial cells of collecting ducts in the kidney (Fig. 7c). Hepatic necrosis with eosinophilic granules in the cells lining the duct was also noticed (Fig. 7d). Focal inflammatory cells infiltration was detected in between the hyalinized bundles of skeletal muscles (Fig. 7e). Inflammatory cells infiltration and granular eosinophils with dilatation in the blood vessels were detected in the base of the gill filaments (Fig. 7f), while the arch had also focal hemorrhages with dilatation in the blood vessels. The rakers showed massive number of goblet cells formation (Fig. 7g).

Residues

Detection of residues in the muscles of Tilapia nilotica exposed to $^1/_{10}$ LC_{50} /96hr.of mercuric chloride revealed, increase of the amount of residue (ppm/gm) in comparison to the control. Values were 1.1320, 1.7140, 2.3620 and 3.5640 ppm/g at the 2nd, 4th, 6th and 8th weeks respectively (Table 2).

Short term exposure to sublethal dose $\frac{1}{2}$ LC_{50}/ 96 hr of copper sulfate in Tilapia

Postmortem findings showed congestion of liver, spleen and yellowish coloration of the body cavity. Histopathological examination in 2nd day showed degenerative changes in renal tubules (Fig. 8a). Melanophors aggregation was seen in the wall of the blood vessels of the spleen (Fig. 8b) with depletion of some of the melanophors in the melanomachro-phage center (Fig. 8c). There were granular degeneration and vacuolization in the sarcoplasm of the skeletal muscles (Fig. 8e). Massive number of granular eosinophilic cells were detected in the base of the filaments (Fig. 8f), associated with hemorrhages and dilatation in the blood vessels in the arch (Fig. 8g). In 5th day liver showed congestion of blood vessels, vacuolation of hepatocytes (Fig. 9a), areas of hemorrhage and melanophores vacuolar degeneration (Fig.s 9b). Necrosis was seen in some areas of melanomachrophage center in the spleen (Fig. 9c). There was hyalinization in the skeletal muscle bundles (Fig. 9d). Sever congestion was noticed in the blood vessels of the gill filaments (Fig. 9e) associated with massive number of granular eosinophilic cells infiltration in the base of the filaments (Fig. 9f).

In the 7th day necrosis appeared in some areas of the spleen (Fig.s 10a-10b) and some hyaline droplets appeared in the kidney (Fig. 10c). Sever vacuolization was observed in the skeletal muscle bundles (Fig. 10d). The base of the filaments showed massive number of eosinophilic and inflammatory cells infiltration (Fig. 10e), while the rakers showed massive number of goblet cells formation and focal stratification in

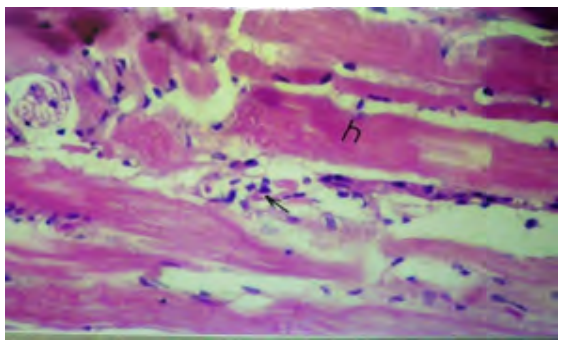

Fig. (4a): Skeletal muscles 2 weeks after exposure to mercuric chloride. Showing few inflammatory cells infiltration in between the bundles (arrow)
H&E (X80)

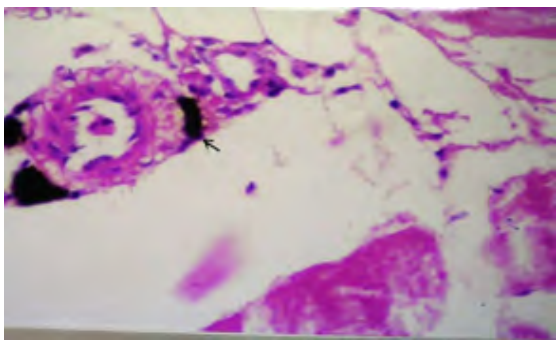

Fig. (4b): Skeletal muscles 2 weeks after exposure to mercuric chloride. Showing focal pigmentation (arrow) in the perivascular area between the bundles.
H&E (X80)

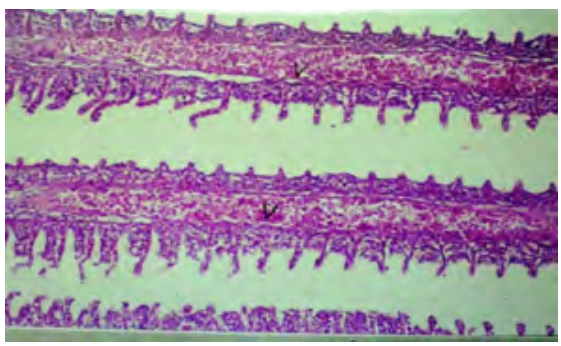

Fig. (4c): Gills 2 weeks after exposure to mercuric chloride showing congestion in the gill filament (v)
H&E (X64)

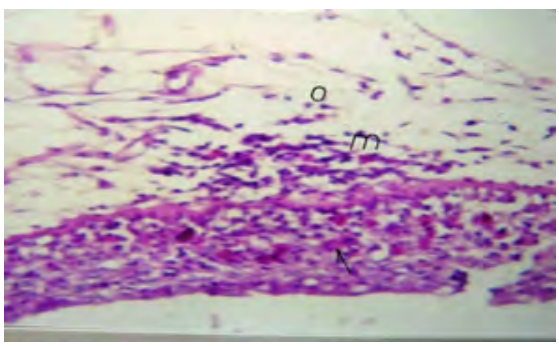

Fig. (4d): Gills 2 weeks after exposure to mercuric chloride. Showing edema (o), eosinophilic cells (arrow) and leucocytes inflammatory cells infiltration (m) in the arch.
H&E (X64)

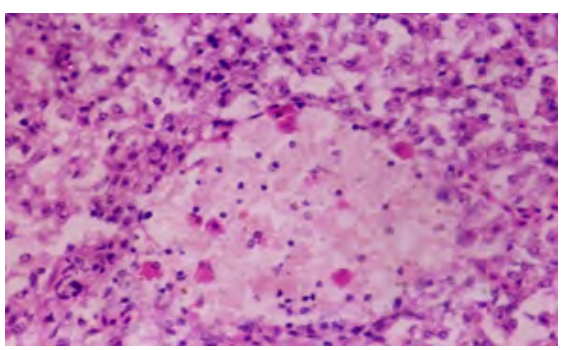

Fig. (5a): Liver 4 weeks after exposure to mercuric chloride notice area of necrosis and eosinophilic granules.
H&E (X400)

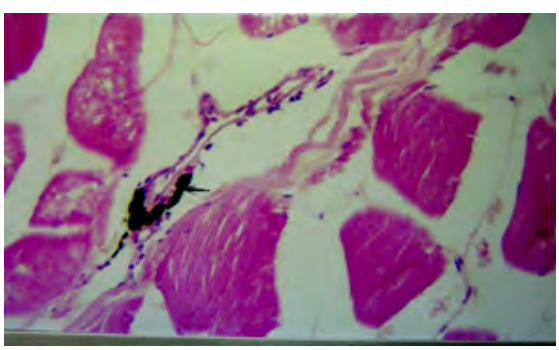

Fig. (5b): Skeletal muscles 4 weeks after exposure to mercuric chloride notice perivascular pigmentation (arrow) in between the bundles.
H&E (X80)

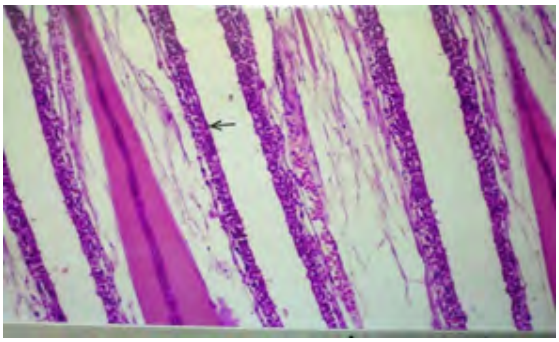

Fig. (5c): Gills 4 weeks after exposure to mercuric chloride notice loss of the lamellae (arrow)
H&E (X40)

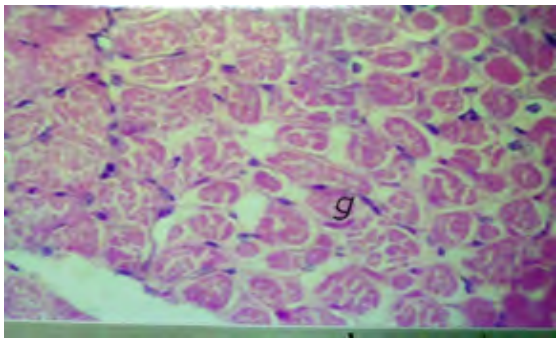

Fig. (6a): Skeletal muscles 6 weeks after exposure to mercuric chloride notice the sarcoplasm of the bundles showed granular degeneration (g)
H&E (X64)

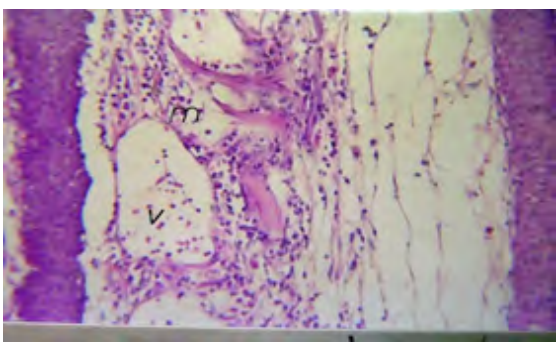

Fig. (6b): Gills 6 weeks after exposure to mercuric chloride showing inflammatory cells infiltration (m) with dilatation in the blood vessels (v) in the gill arch.
H&E (X40)

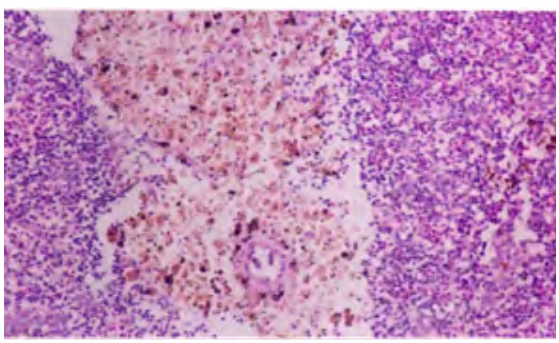

Fig. (7a): Spleen 8 weeks after exposure to mercuric chloride showing hyperactivity in melanomachrophage center.
H&E (X200)

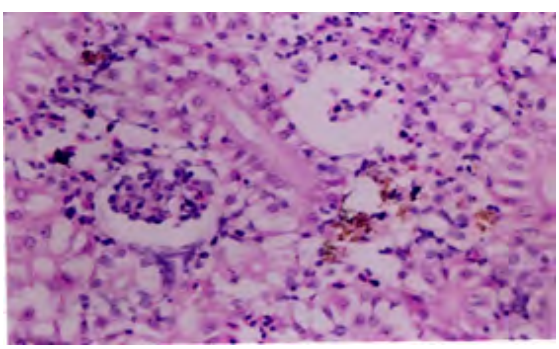

Fig. (7b): Kidney 8 weeks after exposure to mercuric chloride showing glomerular atrophy and necrosis.
H&E (X400)

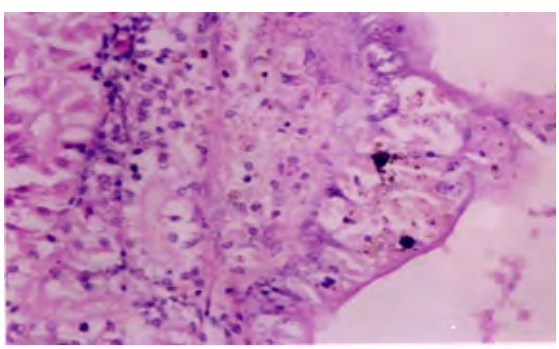

Fig. (7c): Kidney 8 weeks after exposure to mercuric chloride showing necrosis and melanophores in the epithelial cells of collecting ducts.
H&E (X400)

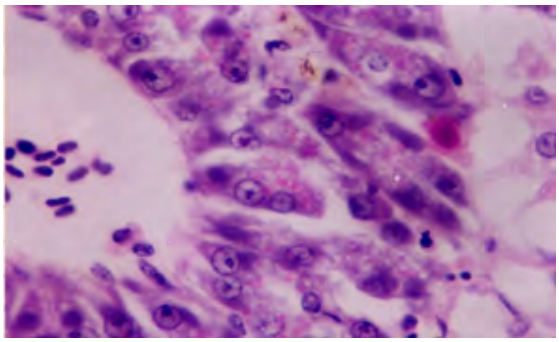

Fig. (7d): Liver 8 weeks after exposure to mercuric chloride showing hepatic necrosis with eosinophilic granules in the cell lining the duct.
H&E (X1000)

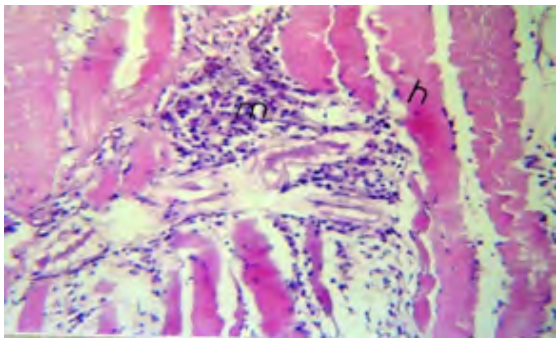

Fig. (7e): Skeletal muscles 8 weeks after exposure to mercuric chloride showing focal inflammatory cells infiltration (m) was detected in between the hyalinized bundles (h).
H&E (X64)

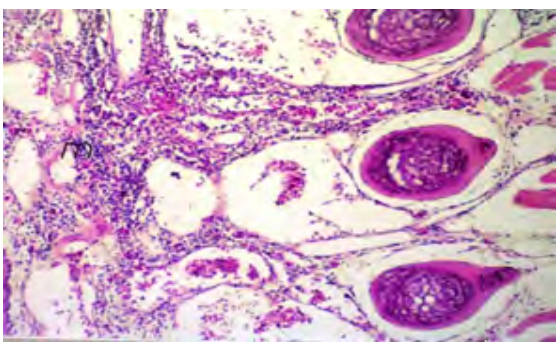

Fig. (7f): Gills 8 weeks after exposure to mercuric chloride showing inflammatory cells infiltration and granular eosinophils (m) with dilatation in the blood vessels (v) in the base of the filaments
H&E (X64)

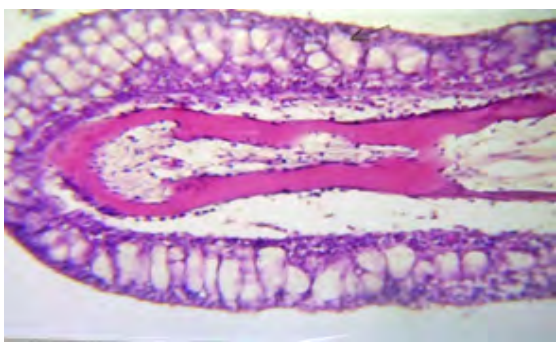

Fig. (7g): Gills 8 weeks after exposure to mercuric chloride notice the rakers showed massive number of goblet cells formation (arrow)
H&E (X64)

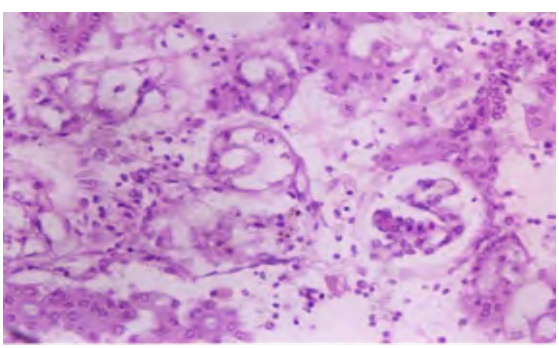

Fig. (8a): Kidney 2 days after exposure to copper sulfate showing degenerative changes of renal tubules.
H&E (X400)

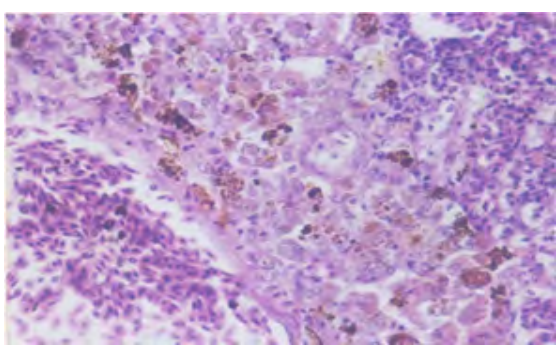

Fig. (8b): Spleen 2 days after exposure to copper sulfate showing melanophores aggregation in the wall of the blood vessels.
H&E (X400)

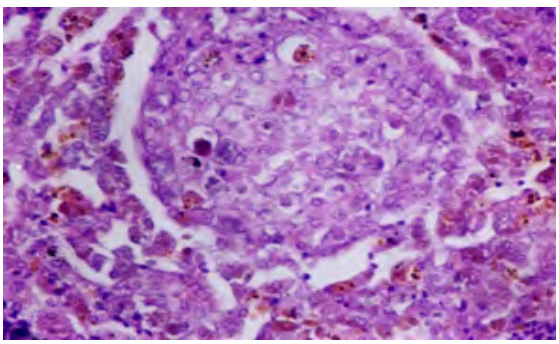

Fig. (8c): Spleen 2 days after exposure to copper sulfate notice depletion of melanophores in the melanomachrophage center.
H&E (X400)

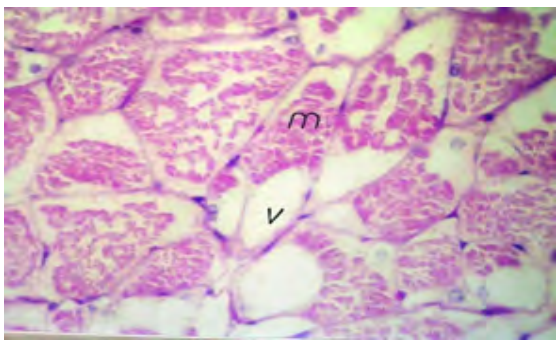

Fig. (8d): Skeletal muscles 2 days after exposure to copper sulfate showing granular degeneration (m) and vacuolization (v) in the sarcoplasm.
H&E (X160)

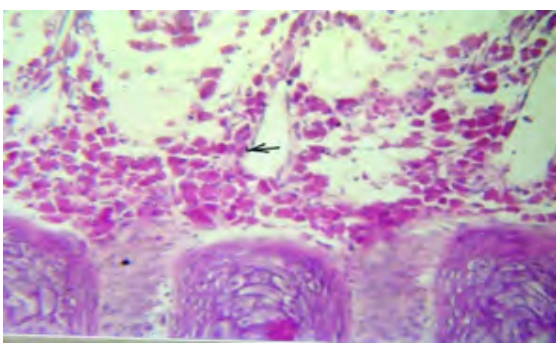

Fig. (8e): Gills 2 days after exposure to copper sulfate showing massive number of granular eosinophilic cells in the base of the filaments (arrow).
H&E (X64)

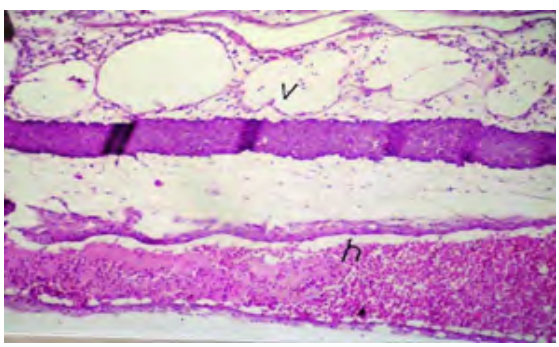

Fig. (8f): Gills 2 days after exposure to copper sulfate showing hemorrhages (h) with dilatation in the blood vessels in the arch (v).
H&E (X40)

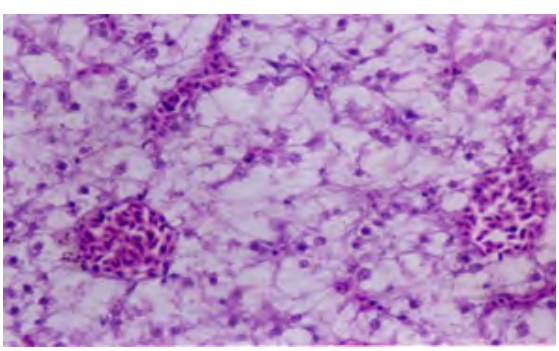

Fig. (9a): Liver 5 days after exposure to copper sulfate showing congestion of blood vessels and vacuolation of hepatocytes.
H&E (X400)

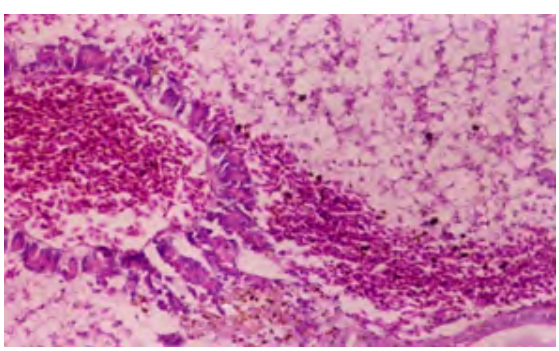

Fig. (9b): Liver 5 days after exposure to copper sulfate showing area of haemorrhage and melanophores vacuolar degeneration.
H&E (X200)

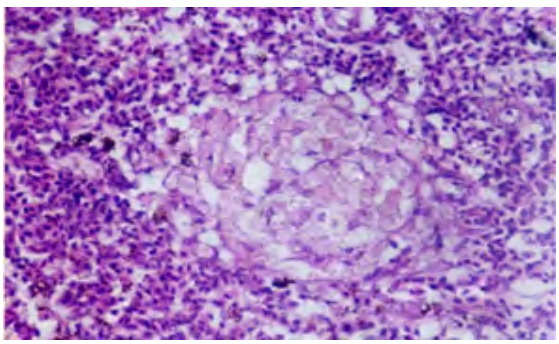

Fig. (9c): Spleen 5 day after exposure to copper sulfate notice necrosis of some areas of melanomachrophage center.
H&E (X400)

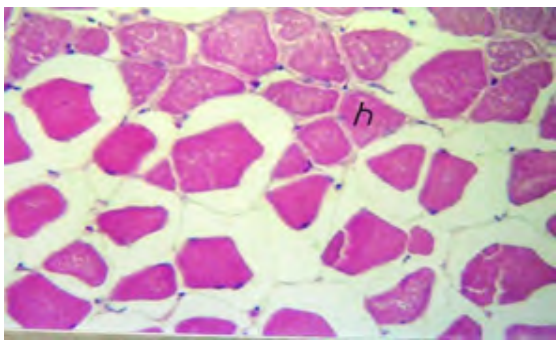

Fig. (9d): Skeletal muscles 5 days after exposure to copper sulfate notice hyalinization in the bundles.
H&E (X80)

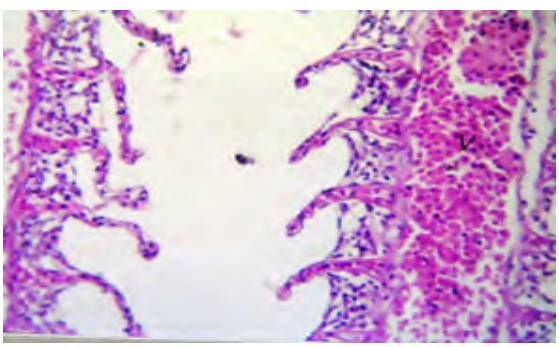

Fig. (9e): Gills 5 days after exposure to copper sulfate notice sever congestion in the blood vessels of the gill filaments.
H&E (X80)

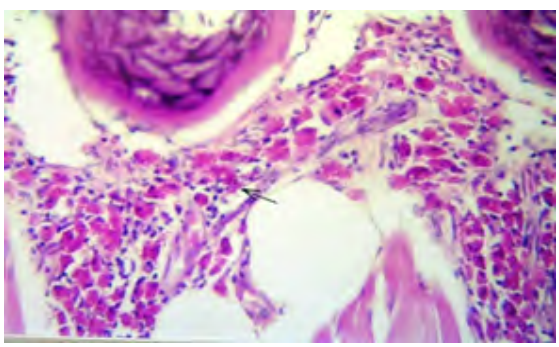

Fig. (9f): Gills 5 days after exposure copper sulfate. Showing massive number of granular eosinophilic cells infiltration in the base of the filaments (arrow).
H&E (X80)

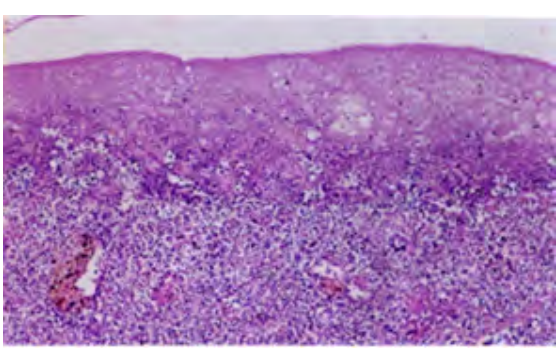

Fig. (10a): Spleen 7 days after exposure to copper sulfate showing necrosis in some areas of the spleen.
H&E (X100)

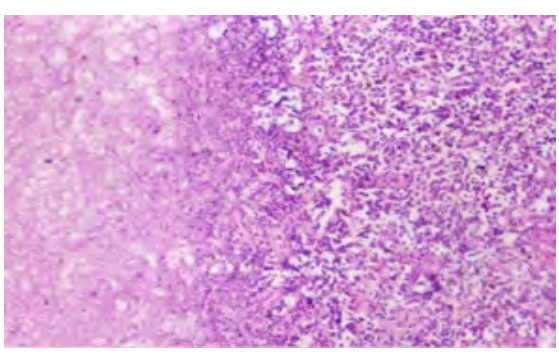

Fig. (10b): Spleen 7 days after exposure to copper sulfate showing necrosis in some areas of the spleen.
H&E (X200)

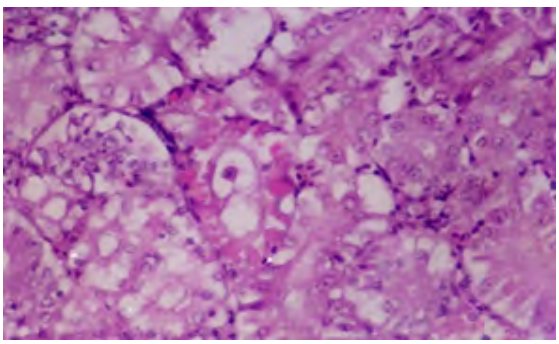

Fig. (10c): Kidney 7 days after exposure to copper sulfate notice hyaline droplets.
H&E (X200)

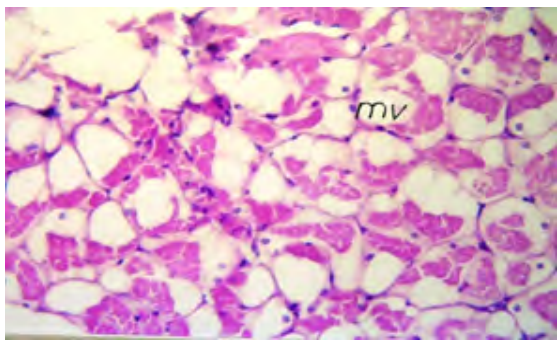

Fig. (10d): Skeletal muscles 7 days after exposure to copper sulfate notice sever vacuolization in the bundles.
H&E (X80)

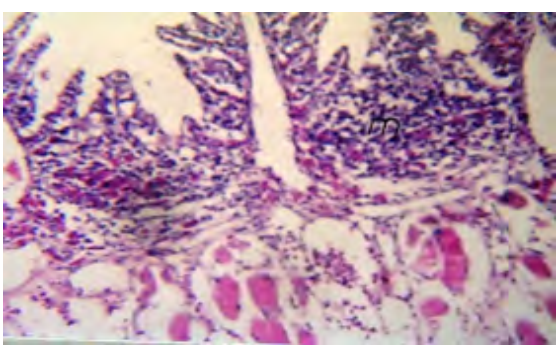

Fig. (10e): Gills 7 days after exposure to copper sulfate notice massive number of inflammatory cells infiltration (m) and eosinophils in the gill filaments.
H&E (X64)

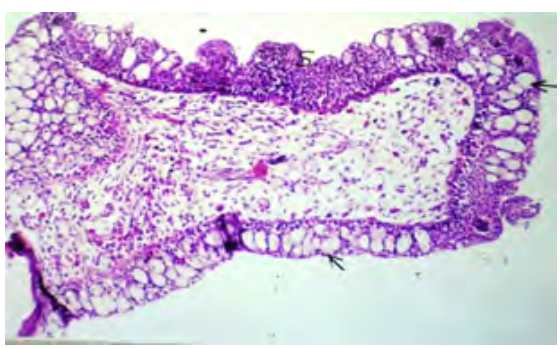

Fig. (10f): Gills 7 days after exposure to copper sulfate showing massive number of goblet cells formation (arrow) and focal stratification in the covering cells of rakers.
H&E (X40)

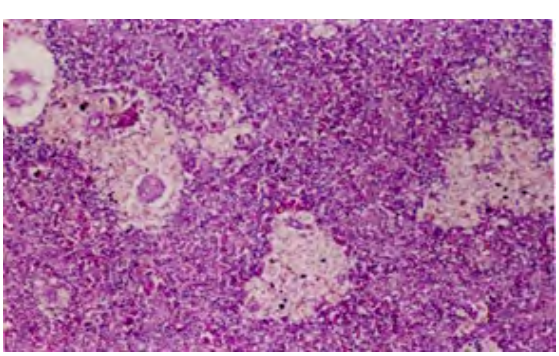

Fig. (11a): Spleen 2 weeks after exposure to copper sulfate showing necrosis in melanomachrophage center.
H&E (X100)

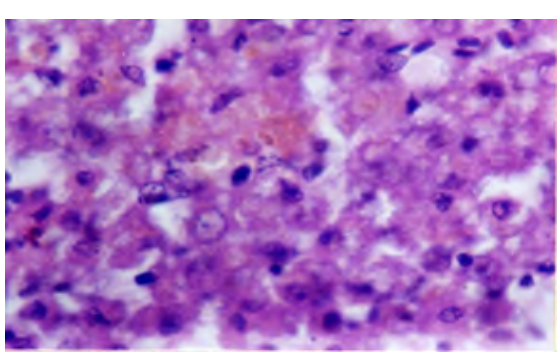

Fig. (11b): Spleen 2 weeks after exposure to copper sulfate showing fragmentation of the nucleus.
H&E (X1000)

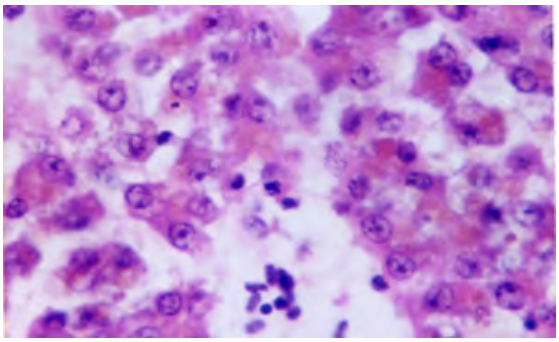

Fig. (11c): Liver 2 weeks after exposure to copper sulfate notice mitosis in some areas.
H&E (X100)

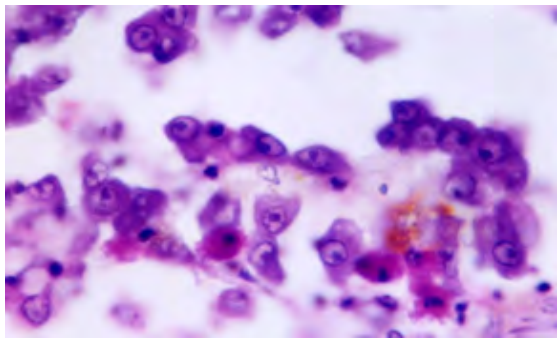

Fig. (11d): Liver 2 weeks after exposure to copper sulfate Notice displasia of hepato pancreatic cells and eosinophilic granular cells aggregation.
H&E (X1000)

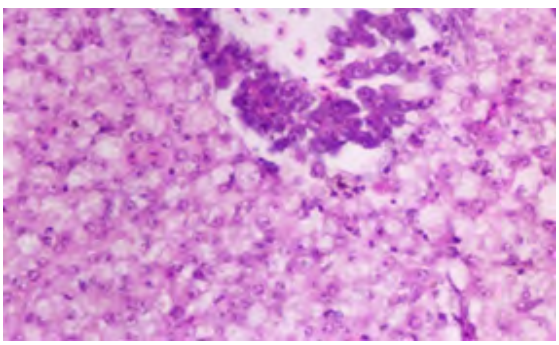

Fig. (11e): Liver 2 weeks after exposure to copper sulfate notice disappearance of zymogenic granules, granular and dysplasia of hepatocytes.
H&E (X400)

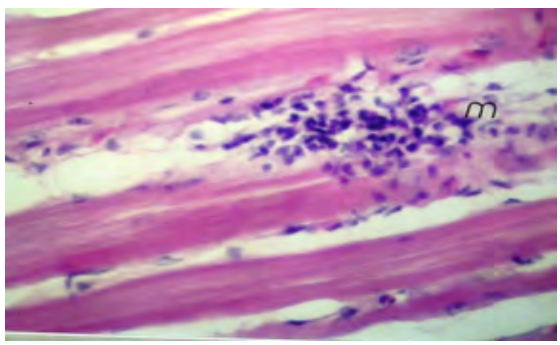

Fig. (11f): Skeletal muscles 2 weeks after exposure to copper sulfate showing focal inflammatory cells infiltration (m) in between the bundles.
H&E (X160)

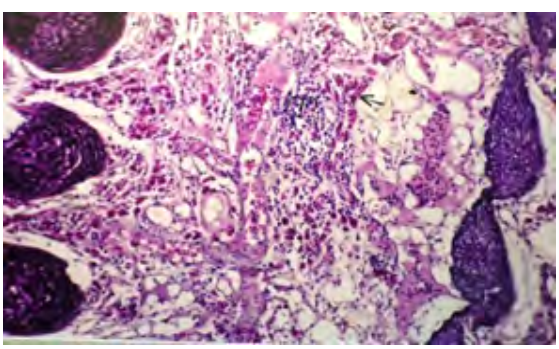

Fig. (11g): Gills 2 weeks after exposure to copper sulfate notice the arch showed eosinophilc (arrow) and inflammatory cells infiltration (m).
H&E (X40)

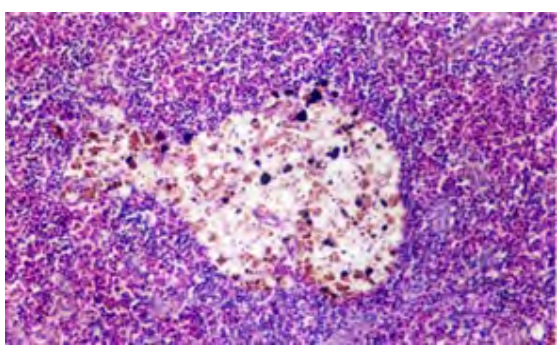

Fig. (12a): Spleen 4 weeks after exposure to copper sulfate notice degenerative changes in the spleen.
H&E (X200)

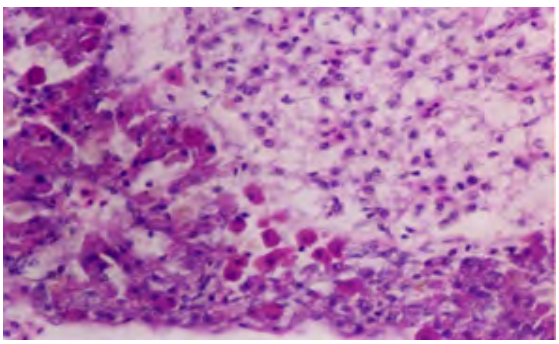

Fig. (12b): Liver 4 weeks after exposure to copper sulfate showing eosinophilic granule cells at the area of hepatopancreas.
H&E (X400)

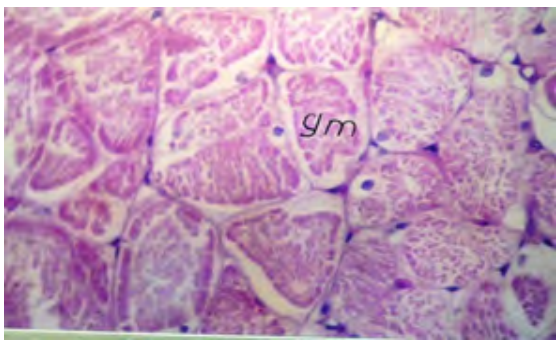

Fig. (12c): Skeletal muscles 4 weeks after exposure to copper sulfate showing granular degeneration (gm) in the sarcoplasm of the bundles.
H&E (X160)

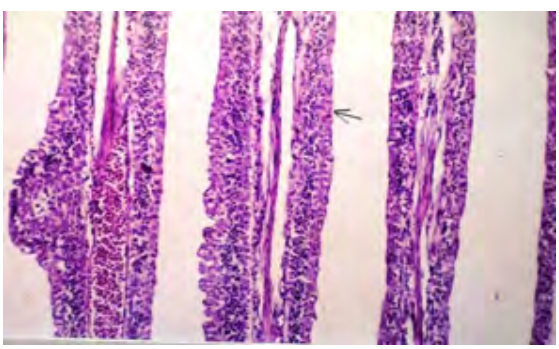

Fig. (12d): Gills 4 weeks after exposure to copper sulfate notice the filaments lost their lamellae (arrow).
H&E (X40)

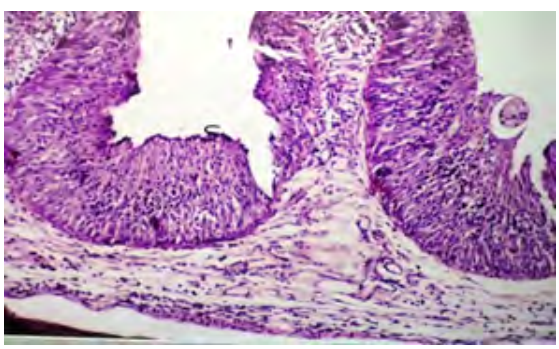

Fig. (12e): Gills 4 weeks after exposure to copper sulfate showing sever stratification (s) in the rakers.
H&E (X40)

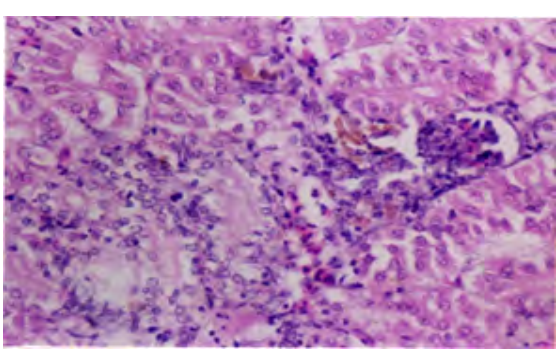

Fig. (13a): Kidney 6 weeks after exposure to copper sulfate showing aggregation of melanophores.
H&E (X400)

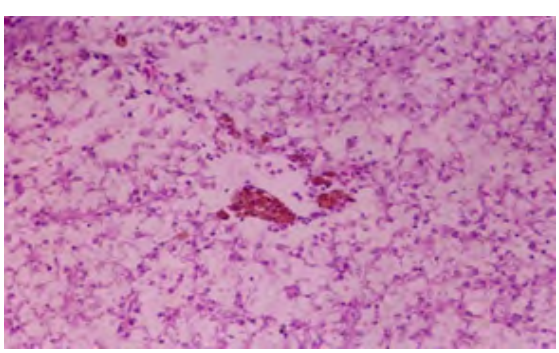

Fig. (13b): Liver 6 weeks after exposure to copper sulfate showing necrosis in melanomachrophage center.
H&E (X100)

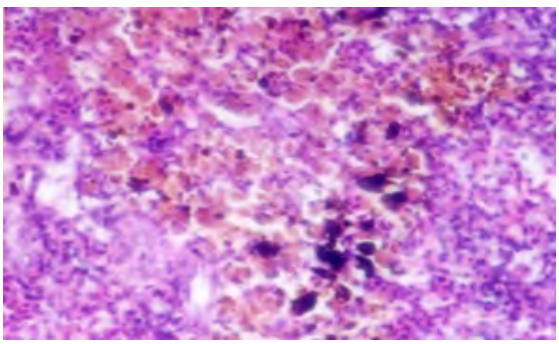

Fig. (13c): Spleen 6 weeks after exposure to copper sulfate showing areas of degenerative changes.
H&E (X400)

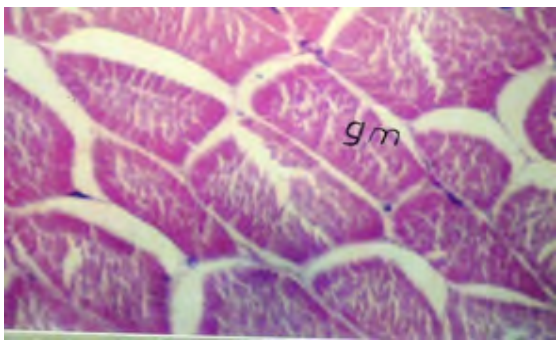

Fig. (13d): Skeletal muscles 6 weeks after exposure to copper sulfate showing granular sarcoplasm with swelling (gm) in the bundles.
H&E (X160)

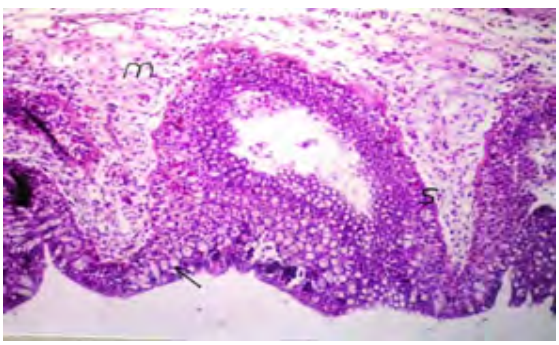

Fig. (13e): Gills 6 weeks after exposure to copper sulfate. showing stratification (s) in the rakers with goblet cells formation (arrow) and inflammatory cells infiltration in the underlying tissue (m).
H&E (X40)

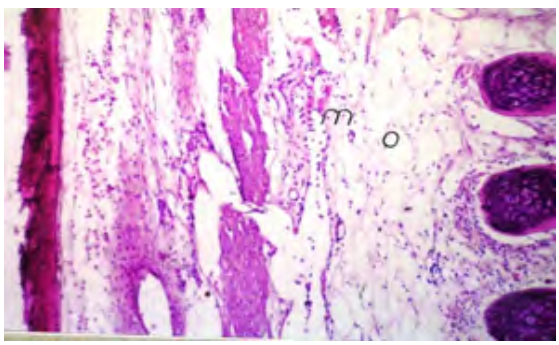

Fig. (14a): Gills 8 weeks after exposure to copper sulfate notice oedema (o) with few inflammatory cells (m) infiltration in the arch.
H&E (X40)

the covering cells (Fig. 10f).

Residues

Detection of residues revealed increase in the amount of copper measured as ppm/gm in comparison to the control. Copper value were 0.60, 0.67 and 0.67 ppm / gm at the 2nd , 5th and the 7th day respectively (Table 1).

Long term exposure to sublethal dose ($^{1}/_{10}$ LC $_{50}$/ 96 hr.) of copper sulfate in Tilapia nilotica fish

Postmortem findings showed congestion of some parts of the kidney, liver and spleen together with yellow pigmentation in some organs and in the body cavity.

In 2nd week post exposure necrosis appeared in the melanomachrophage center (Fig. 11a) and fragmentation of the nucleus of spleen were seen (Fig. 11b). Liver sections showed mitosis in some areas (Fig. 11c) with displesia of hepatopancreatic cells and eosinophilic granular cells aggregation (Fig.s 11d). Zymogenic granules disappeared and there was dysplasia of hepatocytes (Fig. 11e). There was focal inflammatory cells infiltration inbetween the bundles of the skeletal

muscles (Fig. 11f). The gill arch showed eosinophilic and inflammatory cells infiltration (Fig. 11g).

Results in 4th. week post exposure showed degenerative changes in the spleen (Fig. 12a). Hepatic tissues revealed eosinophilic granule cells at the area of hepatopancreas (Fig. 12b). Glomerular atrophy in renal tissue together with necrosis were observed. Granular degeneration was observed in the sarcoplasm of the skeletal muscle bundles (Fig. 12c). The gill filaments lost their lamellae (Fig. 12d), and the rakers showed sever stratification (Fig. 12e).

In 6th. week post exposure renal tissue revealed same lesions as in the 4th week, accompanied with focal aggregation of inflammatory cells and melanophores (Fig. 13a). Necrosis in melanomachrophage center of the liver was seen (Fig. 13b) as well as degenerative changes in some areas of the spleen (Fig. 13c). Skeletal muscle bundles showed granular sarcoplasm with swelling (Fig. 13d). Stratification was noticed in the gill rakers with goblet cells formation (Fig. 13e).

In 8th. week post exposure the same lesions were observed as in the 8th week. No histopathological alterations were recorded in the skeletal muscles .Oedema with few inflammatory cells infiltration were detected in the gill arch (Fig. 14a).

Residues

Detection of residue revealed increase in its amount in comparison to the control. It was 0.18, 0.18, 0.19 and 0.21 ppm/gm at the 2nd, 4th, 6th and the 8th week respectively (Table 2).

Discussion

Histopathological findings in acute and chronic mercuric chloride toxicity revealed degeneration and necrosis in the glomeruli, interstitium tissue and epithelium lining renal tubules. The tubular epithelium became necrotic at several places. Eosinophilic hyaline droplets is released in the cytoplasm of cells suffered from necrosis. Degenerative changes in the melanomachrophage center were also recorded. Hyperactivity in melanomachrophage center was seen in the spleen together with some necrotic areas. Hepatic cells showed necrosis and aggregation of melanomachrophage cells, vacuolar degeneration in the hepatic cells. Gills showed loss in the lamellae of the filaments associated with edema, inflammatory cells infiltration and haemorrhages in the arch. The sarcoplasm of the bundles of the skeletal muscle showed granular degeneration and focal inflammatory cells infiltration between the hyalinized bundles .These results agree with Studnicka (1983), George et al. (1995), Kotsanis and Lliopoulou (2001), Oliveira et al. (2002)and Gupta and Kumar (2006).

The central idea for histopathological studies involving Hg is that the metal induces necrotic changes in virtually every organ and tissue evaluated histopathologically. Mercury tenaciously binds sulfhydryl groups on the plasma membrane and other cellular constituents (Olson et al. 1973 b and Bouquegneau, 1977) ultimately destroying the permeability characteristics of the cells involved (Trump et al,. 1975 and Lock et al., 1981).

Mercury residues obtained from these studies in the acute toxicity were 0.22 ppm/gm in the 2nd day, 0.411 ppm/ gm in the 5th day ended with 0.96 ppm/ gm in the 7th day. In chronic toxicity it was 1.1320, 1.7140, 2.3620 and 3.5640 ppm/gm respectively from the 2nd to the 8th week of exposure.

Our result vary from others due to many reasons: there is relationship between mercury accumulation and pH and water hardness, gender and form of mercury.

Fish mercury levels were higher at lower pH (Wren and MacCrimmon, 1983).

The uptake of MeHg by rainbow trout was less in hard water (Rodgers, 1982).

In some species of fish, males have been found to harbor higher mercury levels than females (WHO 1989). Our results agree with El-Nabawi et al. (1987)

and Barak and Mason (1990a) who reported mercury level in muscles ranged from 0.08- 0.20ppb. The intestinal wall in fish is an effective barrier to mercury chloride but is permeable to methyle mercury(MeHg) which can accumulate preferentially over time in muscle tissue (about 50% of the total dose) (WHO 1989). Mercury limit recommended by the FAO/WHO and WHO in edible parts of fish samples is 0.5 μg/g. Also Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives has estimated a permissible tolerable weekly intake for MeHg of 3.3 pg/kg/week equivalent to 4.7×10^{-4} mg/kg/day (0.47 pg/kg/day). The U.S. Environmental Protection Agency (U.S. EPA) has derived a reference dose for MeHg which equals 3×10^{-4} mg/kg/day (0.3 pg/kg/day) (U.S. EPA. Iris 1992). For a generic 70-kg adult, this is equivalent to a daily intake of 21 pg/day, and for a 62-kg woman, this dose is equivalent to an intake of 19 pg/day. Recently, RfD (Reference Dose) of 0.07 pg/kg/day was suggested by Stern (1993) based on developmental effects.

The LC_{50} of copper sulfate is recorded to be 0.759 mg/L in Tilapia nilotica fish. The determined LC_{50} in the present study was close to Svecevicius and Vosylien (1996) and Bagdonas and Vosyliene (2006) who found that the 96-hour LC_{50} of copper was 0.65 mg/L in Oncorhynchus mykiss and also with Ramesh et al. (2007) who found that 24-h LC_{50} value of copper sulfate in fingerlings of scale carp, Cyprinus carpio was 0.7 ppm. The data also agreed with that of Olaifa et al. (2004) who reported that 96-hour LC_{50} in Clarias gariepinus projected using the method of logarithm were 0.6, 0.71 and 0.7 mg/L for imitates 1, 2 and 3 correspondingly with a mean value of 0.67 mg/L. Emad et al. (2005) found that 96-h LC_{50} of copper sulfate was 1.64 ppm in fingerlings of marine fish Mugil seheli.. Our results disagree with Sharif et al. (2001) who reported that 96 -h LC_{50} of copper in Javanese carp Puntius gonionotus was 0.53 mg/L. Mazon and Fernandes (1999) calculated 29 μg Cu/L–1 as the LC_{50} of copper for Juvenile Prochilodus scrofa. Ann et al. (1995) recorded 175 ppb LC_{50}/96hr. of copper sulfate in gold fish Carassius auratus. This difference in the toxicity might be due to the differences in fish species and degree of affection by the temperature, Ph and water hardness as well as the size of the fish. Copper is more toxic to small fish than to large fish. This may be due to the reduced metabolic rate per g of fish (Lauren and McDonald, 1986).

Histopathological examination showed, in acute and chronic copper toxicity, degenerative changes in renal tubules. Melanophores aggregation in the wall of the blood vessels of the spleen and depletion of some of the melanophores in the melanomachrophage were seen together with necrosis in some areas. Congested Mvs (Micro vessels) and vacuolation of hepatocytes were observed. Some areas of hemorrhage and melanophores vacuolar degeneration in the liver were seen. Examination also showed mitosis in some areas with displesia of hepatopancreatic cells and eosinophilic granular cells aggregation. Zymogen granules disappeared and there were dyplastic hepatocytes. Congestion in the blood vessels of the gill filaments, associated with massive number of granular eosinophilic cells infiltration were seen in the base of the filaments. There were sever vacuolization and hyalinization in the skeletal muscle bundles. Our results come in agreement with Williams and Wooten (1981), Sultan and Khan (1983), Asztalos et al. (1990), Mohey (1996), Zsolt et al. (2001), Koca et al. (2005) and Fig.ueiredo et al. (2007) who found different histopathological changes in different species of fish after copper exposure.

Detection of residues of copper sulfate in Tilapia nilotica fish exposed to acute copper toxicity revealed increase of the amount of copper measured in ppm/gm comparing to the normal control starting from 0.60 ppm/g in the 2nd day, 0.67 ppm/g in the 5th day and 0.67 ppm/g in the 7th day. Result obtained in chronic copper sulfate toxicity revealed gradual increase of the amount of copper which ranged from

0.18 ppm/g at the 2nd week to 0.21 ppm/g in the 8th week of exposure. Our result agree with Adeyeye and Emmanuel (1994), Manal (1995), Wong et al. (1999) and Wannee and Niwa (2007) who reported different concentrations in muscle tissues after exposure to copper as the accumulation was dose dependent. Extoxnet (1996) explained that the absorption of copper sulfate into the blood occurs primarily under the acidic conditions of the stomach. The mucous membrane lining of the intestine acts as a barrier to absorption of ingested copper. After ingestion, more than 99% of copper is excreted in the feces. However, residual copper is an essential trace element that is strongly bio-accumulated. It is stored primarily in the liver, brain, heart, kidney, and muscles.

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