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Ameliorative effect of taurine and sawdust on copper sulphate toxicity in *Oreochromis niloticus* with referring to public health hazards of its residues

Rehab, E. Mowafy[*]; Hend, M. Megahed[**]; Mervat A. Ayoub[*]; Amina M. El-Amin[***] and Mohamed A. Bakry[****]

Pathology[*]; Biochemistry[**]; Food hygiene[***] and Fish Diseases and Management [****]Dep. Animal health research institute (Zagazig branch) Agriculture Research Center (ARC), Egypt.

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Abstract

The present study was executed to evaluate the ameliorative effect of both taurine and chemically treated sawdust powder as two models (one are chemical and other are physical adsorbent respectively) on copper sulphate intoxicated ***Oreochromis niloticus***. An experiment was applied on ninety fish divided into six equal groups treated consequently with non (control),copper sulphate not treated ,copper sulphate treated with taurine, copper sulphate ,treated with sawdust powder, taurine and finally sawdust powder, all for a month. Our work was supported by an early survey study implemented in some fish markets proved presence of 14% of the examined *oreochromis niloticus* musculature exceeded maximum permissible limit of copper .Pathological, biochemical and residual studies for detection of copper toxicity were done. Pathological results showed the most prominent alterations grossly and microscopically in fish gills, livers and muscles, congestion, deformities in the secondary lamellae with telangectiasis with leucocytic cells infiltrations in all examined organs, vacuolation of the hepatic cells. Milder lesions appeared in the examined organs of the third and the fourth group were exhibited due to applying both taurine and chemically treated sawdust powder. Biochemical results revealed that chronic exposure of copper sulphate in fish induced marked alterations in Hb, RBC, PCV, total leucocyte count, total protein and antioxidants enzymes (GPX, CAT and SOD) compared to control, while taurine and sawdust powder treated groups revealed significant improvement of these parameters and reduced copper sulphate accumulation in biological tissues of fish intended for human consumption. The results suggest that taurine and sawdust powder have potential effects to relieve the copper toxicity in ***Oreochromis niloticus*** and to decrease the accumulation of copper percent in the *oreochromis niloticus* musculature and therefore toxicity in fish consumers.

Keywords: *Taurine, Sawdust powder, Copper sulphate, Oreochromis niloticus.*

Introduction

Oreochromis niloticus is one of the most plentiful seated fish present in the most Egyptian Lakes, Nile River, and Ponds, considered a standout amongst the most evaluative freshwater species for its God gifted strong immune system which maximizes their capability to tolerate biotic and abiotic types of stress **(Girón-Pérez *et al.*, 2007)**. ***O.niloticus*** also considered as bio-indicator in understanding environmental pollution involved in determining the level of heavy metal in aqua culture **(Firat and Kargin, 2010).** Consequently this allow Egypt to be the world's second largest producer of farmed tilapia after China (**Rothuis *et al*., 2013).**

Heavy metals are considered one of the most hazardous and harmful metals even in trace amount, since they accumulate in the tissue of living organisms **(Rao *et al.,* 2010).** In the aquaculture industry, copper sulfate is used as a therapeutic chemical for various ectoparasitic and bacterial infections**) Nouh and Selim, 2013 and Lasiene *et al.,* 2016).** Increasing agricultural production leads to increase the number of freshwater systems being impacted by the contaminants present in wastewater releases like copper sulphate which used extensively as a fungicide, so high concentration of copper sulphate was detected in some aquatic ecosystems found in freshwater **(Atabati *et al.*, 2015).**

The toxic effect of copper is due to its capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species **(Lopes *et al.*, 2001)**, also haemolytic anaemia, a common complication of copper sulphate poisoning, is caused either by direct red blood cell membrane damage **(Chuttani *et al.*, 1965)** or indirectly as a result of the inactivation of enzymes which protect against oxidative stress **(Mital *et al.,* 1966).** Copper can act as sodium analogues and competitors in gill transport systems, and out-compete sodium, thereby blocking transport systems **(Grosell and Wood, 2002)**. Fish are particularly sensitive to water contamination and pollutants may impair many physiological and biochemical processes in fish tissue **(Durmaz *et al.,* 2006)**. Copper is a powerful oxidant causing inflammation and free radical damage to the tissues. To avoid these toxic effects, it must be bound to the binding proteins, ceruloplasmin and metallothionein. These proteins can become deficient due to impaired adrenal and liver function which allows free copper to build up **(Sinkovic *et al.*, 2008).** It can have a toxic effect on the body and mind and it is a contributor to many chronic illnesses and mental disturbances.

Taurine (2-amino ethanesulfonic acid) is a derivative of a sulfur-containing amino acid and is a conditionally essential nutrient and the major free intracellular non protein sulphur amino acid **(Atmaca, 2004)**. I's derived from methionine and cysteine metabolism, present in high concentration in most tissues particularly in pro-inflammatory cells such as polymorphonuclear phagocytes **(Droge and Breitkreutz, 1999)**. Taurine interacts directly with metal ions by electric association between metal cations and the sulfonate anion or by the interaction between metal ions and the nitrogen's unshared pair of electrons **(Wright *et al.,* 1985).** Taurine is reported to form less stable metal complexes with various transition metals such as copper ions, nickel ions, zinc ions,…etc , compared to other amino acids **(Flora *et al.,* 2008).** Taurine has antioxidative action attributed to its ability to stabilize biomembranes **(Han *et al.*, 2014)** and scavenging reactive oxygen species **(Wright *et al.,* 1985).**

Pathological alterations were observed after the exposure to copper sulphate were mainly observed in gills, epithelial hyperplasia, edema in the filamental epithelium, lifting of the lamellar epithelium, Curling, clubbed tips of secondary lamellae and finally a complete fusion of several secondary lamellae at the 35 mg copper sulphate concentration. Moreover, the liver showed histological alternations such as cytoplasmic rarefaction, an increase of cytoplasmic vacuolation, decreasing the number of hepatocytes nucleus in hepatic tissue and nuclear pyknosis. **(Alkobaby and Abd El -Wahed, 2017).**

There are various methods for removing heavy metals including chemical precipitation, membrane filtration, ion exchange, liquid extraction or electro dialysis but these methods are not widely used due to their high cost, In contrast, adsorption is the most effective method has been successfully applied in the purification and recovery of copper ions due to its high efficiency, low cost, simplicity and easy handling **(Kurniawan *et al.,* 2006).** Many proceedings have been developed for the effective removal of heavy metals using ,saw dust, walnut and cotton seeds hull **(Memon *et al.,* 2008).**

The objective of the present study was to describe the toxicity status of copper sulphate in *oreochromis niloticus* and to determine ameliorative effect of both taurine and sawdust powder as chemical and physical antitoxic substances consequently in copper toxicity with regarded to pathological and biochemical assessment and also scan the percent of accumulated copper in experimental *O. niloticus* samples compared with those randomly collected from Zagazig fish markets in addition to comparing the results with maximum permissible limits authorized by **E.O.S.Q.C. (2005)** .

Materials and Methods

I-Pre-experimental work

Specimen collection: A total of **50** ***Oreochromis niloticus*** were collected randomly from different fish markets in zagazig city at El - Sharkia governorate, each sample was transferred in a separate sterile and labeled plastic bag to the laboratory under complete aseptic conditions without delay.

Fish analysis: The level of copper in the fish musculature was measured according to **Ghazaly (1988).** The selected pieces of musculature, dried at **105°C** for 48hrs and then grounded to a fine powder. The dried samples were digested according to the method of one gram dry powder was digested in digestive solution of conc. nitric and perchloric acid (**5ml+5ml**). The samples were heated on a hot plate at **800 - 900°C** until they become clear. After cooling the solutions were filtered and the filtrates were adjusted to 50 ml with deionized distilled water. The copper metallic ions were analyzed in fish muscle using SHIMADZU. Atomic Absorption Spectrophotometer Model AA-6800 equipped with flame unit and auto-sampler SHIMADZU ASC–6100. Results were expressed in µg/g dry weight of the tissue.

II-Experimental fish and design:

A total of apparently healthy **90** ***Oreochromis niloticus*** weighing **65±5**g were obtained from Centeral Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. For acclimation to laboratory aquaria and condition with 12 h dark 12 h light cycles, pH range of 6.95 to 7.60 and temperature at **25 ± 2°**C, fish were placed into 160 liters glass aquaria capacity with 120 liters net water capacity in our laboratory supplied with aerated de-chlorinated fresh water for one week prior to the experiment. Fish were divided into six equal groups, control one without any special treatments, while **2nd, 3rd and 4th** groups exposed to **8 ppm** of copper sulphate in water for a month**,** copper sulphate toxicity dose and experimental duration were according to **(Wani & Sikdar, 2014),** while **5th and 6th** groups not exposed to copper sulphate toxicity. Both the third and fifth groups were fed on balanced synthetic basal diet mixed with taurine at a dose of **5ppm** for a month **(Kumar *et al.*, 2009)**, while fish of both the fourth and six groups subjected to chemically treated sawdust powder in water at level of **0.5 g / 50 ml** copper sulphate for a month **(Larous and Meniai, 2012).** Our experiment kept an additional aquaria with the same condition, fish number and treatment of the second group for compensating the mortalities that may affecting sampling collection and so statistical analysis. Experimental design was demonstrated in table (1).

Chemicals:

Taurine was purchased from Sigma (Egyptian International Center for Import, Cairo, Egypt) at a dose of **5ppm** for a month.

Copper sulphate (CuSO4): Copper sulfate was purchased from Sigma (Egyptian International Center for Import, Cairo, Egypt**.** In a dose of 8 ppm copper sulphate concentrations **(Wani & Sikdar, 2014)**.

Sawdust: with mesh size of **125–200** ml was mixed well with the required amount of NaOH (**0.246**mol/g of sawdust) in a **100** ml Erlenmeyer flask. The resulting reaction mixture was maintained in a thermostatic water-bath at a specified temperature and reaction time. After completion of the reaction, the resulting mixture was neutralized by treatment with acidified ethanol, the precipitated product being washed several times with distilled water to remove the non-reacted Sawdust, followed by washing with acetone and then dried in an electric oven at 333 K for 3h. at level of **0.5 g / 50 ml** copper sulphate for a month **(Larous and Meniai, 2012).**

Blood samples

Five blood samples were collected from each group under complete aseptic condition from caudal blood vessels after 30 days (at the end of experiment) two forms of each blood sample were prepared the first was collected on EDTA for hematological examination (1mL), while the second was taken without anticoagulant in a clean and dry centrifuge tube (3mL), left to clot at room temperature and centrifuged at 3000 rpm for 5 min. Serum was collected, labeled, placed in dry clean-capped tubes and frozen at -20°C for biochemical analysis.

The hematological and biochemical study:
Erythrocytes (RBC), White blood cells count

(WBCs) and white blood cell differential were calculated using the method according to **Feldman *et al* (2000).** Hemoglobin (Hb) concentration was estimated according to **Blaxhall & Daisley (1973).** Glutathione peroxidase GPx activity was assayed according to **Miller and Slebodzinska,(1993),** and Catalase (CAT) was calculated according to **Clairborne (1985).** Superoxide dismutase **(SOD)** was assayed according to **Kakkar *et al.,* (1984).** And serum total protein according to **Tietz, (1995).**

Residual analysis of copper in experimental fish musculature:

Five fish from each group were collected at the end of the experiment for copper residues analysis. All the collected samples subjected to the same analytical method as mentioned before according to **Ghazaly (1988).** Results were expressed in ppm.**(Olaifa *et al*., 2004).**

Histopathological technique:

Tissue specimens were taken from gills, livers and muscles of fish of all experimental groups at the end of experiment and from freshly dead fish and fixed in 10% phosphate buffer formalin. Processed routinely and blocked in paraffin wax then, five micron thick paraffin sections were prepared and stained with hematoxylin and eosin **(Suvarna *et al*, 2013)** for histopathological investigations

Statistical analysis:

Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range **Duncan,(1955)** was used to determine differences among treatments mean at significance level of 0.05. All statistics were run on the computer using the SPSS program **(SPSS, 2004).**

Results and Discussion

Hygienic evaluation of the copper residues in *oreochromis niloticus* musculature.

O.niloticus is one of the fish species considered as bio-indicator species in understanding environmental pollution involved in determining the level of heavy metal in aquaculture. **(Firat and Kargin, 2010).** Moreover, **El-Naggar *et al.,* (2009)** observed that fish could accumulate trace metals and act as indicators of pollution. Results in **table (2)** showed that 14% of ***O. niloticus*** fish samples collected from El Sharkia fish markets contained copper sulphate with concentration exceed the maximum permissible limit stated by the **E.O.S.Q.C. (2005)** which is 20ppm. This result was less than that detected by **Salah-El-Dein *et al*., (2009b)** who found Copper residues within the permissible limit in **23 (76.66%)** out of the examined basa fish, while **7(23.33%)** exceeded this permissible limit in the samples. The elevation of copper accumulation in this study may be due to industrial and sewage wastes. These results agree with those obtained by **Salem, (2003)** who reported that several factors affected the disappearance of some fish species from the River Nile. The first was the High Dam construction and the second was the pollution changes including industrial, drainage and sewage effluents occurs in the Nileriver water **Table. (3)** showed the mean concentration of copper in examined fresh ***O. niloticus*** was **11.15±0.91** ppm. It was lower than the maximum permissible limit allowed in food by **(E.O.S.Q.C. 2005).**

The detected copper levels in the current investigation were higher than those estimated in another Egyptian studies: **Hatem *et al.,* (2003)** detected mean copper in crayfish as (**9.9 ppm**), **Salah-El-dien *et al*., (2008)** had examined copper in tilapia musculature that ranged between **2.9 to 3.8** ppm and **Salah-El- dien *et al.*, (2009a)** who also examined *Clarias gariepinus* along the river Nile and found out that copper concentration ranged from **4.4 to 7.9** ppm. Moreover the obtained copper level was higher than the result obtained by **Cronin *et al.,* (1998)** who reported that copper concentrations of fish from the North East Atlantic ranged between 0.01 and **0.47 mg/kg** and **Mormede and Davies, (2001)** who also reported that copper concentrations in fish musculature ranged between 0.10 and 0.83 mg/kg for fish brought from the North East Atlantic. On the other hand investigations by **Salah-El-Dein *et al*., (2009b)** detected higher mean copper residues reached to (**17.521±2.612**)ppm in the examined pasa fillet fish. **Table (4)** showed that the average concentrations of copper in the examined *o. niloticus* samples gave a daily intake of 1.115 mg/ day /person for fish meat consumers (100 g/ person) and this contribute of about 3.18 %of the acceptable daily intake (ADI) of this metal recommended by **FAO/ WHO (1989). Table (5)** Showed that the mean concentration of copper ions in experimental examined *o. niloticus* musculature after exposure to copper for 30 days in the control group was **23.30±1.29**, this level exceed the maximum permissible limits recorded by the **E.O.S.Q.C. (2005)**, while the examined *o. niloticus* musculuture in the sawdust and taurine treated groups reached **17.46±0.78** and **10.26±0.72** ppm respectively with a reduction % of **25.06%** and **55.96%** respectively this was clearly represented in **figure (I).** This result emphasize the role of sawdust and taurine as a potent adsorbent and antioxidant agents for copper ions. This result is potentiated with what had been reached by **Annad, (2010)** who stated that the efficiency of sawdust powder for the removal of copper from water solutions reached an adsorbent capacity of **30.22** ppm for Copper. Furthermore **Saliba *et al*., (2004)** stated that the treated sawdust showed a suitable proficiency in terms of various heavy metals immobilization such as copper (II), Cadmium (II) and Nickel (II). Also this results agreed with **Kyung-Soon Choi, (2014) and Kumar, (2009)** who mentioned that taurine at different concentrations 20, 40, and 80 helps to minimize the level of heavy metals in examined muscles, gills, and bone tissues of carp fish and **Wani & Sikdar, (2014)** who mentioned that taurine ameliorated the copper induced toxicity in *Clarias gariepinus* due to the great potential of taurine to stabilize the cell membrane and thus could have protected the leucocytes against copper induced toxic damage.

Biochemical results:

The results of hematological parameters presented in **table (6)** revealed that *oreochromis niloticus* exposed to copper sulphate showed a significant decrease of RBCs, Hb and PCV, while, fish received taurine in feed or chemically treated saw dust powder in water showed improvement in values of Hb content, RBC count and PCV in comparison to groups intoxicated with copper sulphate alone. Our findings in accordance with that recorded by **Singh *et al*., (2008**), **Georgieva *et al*., (2010)**, **Nouh and Selim, (2013) and Wani & Sikdar, 2013]**. The decrease in these haematological parameters may be due to impaired intestinal absorption of iron **(Joshi *et al*., (2002)**. or increased destruction of erythrocytes**) Kori-Siakpere *et al.,* 2009)** or heamodilution **(Adeyemo, 2005)** or damage in the hematopoietic system **(Svobodova *et al.,* 1994)**. Regarding to chronic effect of copper sulphate on total leucocyte count and differential leucocyte count, fish exposed to copper sulphate revealed significant decrease in WBCs, lymphocyte, neutrophils and monocytes comparing with negative control group, treatment of fish exposed to copper sulphate with taurine or saw dust resulted in increase in WBCs, lymphocyte ,neutrophils and monocyte. These results parallel to that reported by **Dethloff *et al.,* (2001) & Wani & Sikdar (2014**). These alterations in leucocyte may be attributed to immunosuppression, which occurred due to secretion of cortisol which shortens the life span of lymphocytes and enhances their apoptosis **(Mazon *et al.,* 2002)**, also **Handy, (2003)** mentioned that leucocytopenia incase of copper toxicity due alteration in hemopoietic system. Monocytopenia and neutropenia in our study may be due to disrupted phagocytic activity in the gills, liver and kidney **(Nussey *et al.,* 1995)**. In the present experiment treatment of intoxicated fish with taurine ameliorated the toxic effect of copper sulphate on hematological parameters ,total leucocyte and differential leucocyte count, our results are parallel to that obtained by **[Manna *et al.,* 2008, Anand *et al*., 2010, Wani and Sikdar, 2013 & 2014]**. The improvement in the hematological parameters may be attributed to the ability of taurine to bind to heavy metal by its amino and sulfonate groups and stimulate its excretion, moreover **[Cetiner *et al.,* 2005 and Yeh *et al.,* 2009]** reported that taurine normalized the total leucocyte and differential leucocyte count altered by toxicity. Also, application of chemically treated saw dust powder in fish tank resulted in a significant improvement in hematological parameters, this may be explained by the fact that saw dust contains various organic compounds (lignin, cellulose and hemicelluloses) with polyphenolic groups that could bind heavy metal ions by replacement of hydrogen ions for metal ions in solution or by donation of an electron pair from these groups to form complexes with the metal ions in solution **(Gupta and Ali, 2000)**. In the current study a significant decrease in total protein were detected in intoxicated fish with copper sulphate. This decrease is indicator for impaired liver function **(Chen *et al,* 2002)**. The present results are in harmony with **[Nouh and Selim, 2013 and Mutlu *et al.*, 2015].** Our study revealed that administration of taurine and chemically treated saw dust powder evoked a significant increase in total protein, this result in accordance with **Samipillai *et al.,* (2009)** reported that taurine improved liver functions, as indicated by elevation of serum total protein in rat intoxicated with mercury. In the present study, fish exposed to copper sulphate toxicity and not treated revealed a significant decreases in the antioxidant enzymes levels SOD, GPX and catalase in **table (7)** which indicate impaired antioxidant defense mechanism has a result of the excess production of superoxide radicals by copper and loss of compensatory mechanisms **(Zhang *et al.*, 2004).** Our results in accordance with that recorded by **Gravato *et al.,* (2006), Vutukuru *et al.*, (2006) and Min *et al.*, (2014).** The decline in sodium oxide dismutase (SOD) activity could be due to its inhibition by the excess production of (Reactive oxygen species) ROS. The decrease in (catalase) CAT activity could be due to its inactivation by the superoxide radical or due to declines in the rate of the reaction due to the excess production of H2O2. In the present study a depletion of (Glutathione peroxidase) GPX level in fish exposed to copper sulphate, this in accordance with results reported by **Jena *et al.*, (2009).** The depletion of glutathione may be due to the fact that copper interfere with glutathione synthesis **(Gravato *et al.,* 2006).** In the present experiment fish intoxicated with copper and treated with taurine or sawdust evoked a significant improvment in levels of antioxidant enzymes compared with intoxicated fish, our results in accordance with that reported by **Jagadeesan and Sankar samiPillai (2007) & Manna *et al.*, (2008) and Kumar *et al.*, (2009).** This improvement in antioxidant enzymes may be attributed to the protective effect of taurine against the hepatotoxicity induced by free radicals generating in liver tissues, more over taurine has a direct and indirect antioxidant effect, direct by retrieve oxygen free radicals, thus inhibiting lipid peroxidation and indirect by controlling the increase in membrane permeability resulting from oxidative stress in liver **(Koch *et al.*, 2004)**.

Clinical signs and mortality rate

Clinically, only the copper sulphate treated group showed the most prominent signs as, gasping, reduction of activity mixed with unbalanced fast movement, swimming close to the aquarium surface and copious mucus secretion with loss reflex action were seen till the end of the experiment, these signs were almost similar to those obtained by **Alkobaby and Abd El-Wahed, (2017)**. The mortality rate was **17.77%** (**16 fish**) throughout the experimental period**,** 11from the second group, 4from sawdust powder and copper sulphate treated group and one from taurine and copper sulphate treated group, mortality rate was different than that obtained by **Nouh and Selim, (2013)** who found **5%** mortalities which could be attributed to the differences in dose and time of exposure based on **(Brungs *et al*, 1977**) who mentioned the reaction and survival of aquatic animals depend not only on the biological state of fish and physico-chemical characteristics of water but also on kind, type, toxicity and time of exposure to the toxicant agents. **Groups 3 & 4** showed moderate degree of the previously mentioned signs except absence of copious mucus secretion which could be explained on a base of ameliorative effects of both taurine and sawdust powder against copper sulphate **Ojedokun and Olugbenga, (2015) and Wani and Sikdar, (2014)** clinically, fish of the fourth group showed less improvement when compared with those of the third group which indicate superiority of taurine as antitoxic substance to sawdust powder as adsorbent antitoxic reagent in spite of differences with some references as **Kurniawan *et al.,* (2006)** who described adsorption is the most effective method that has been successfully applied in the purification and recovery of Cu (II) ions from effluents due to its high efficiency and easy handling, while **groups 5 & 6** showed apparently normal behavior and signs seemed to be as control group.

Gross findings:

Group 2 (8ppm Copper sulphate intoxicated group):

The copper sulphate exposed *O. niloticus* showed different degrees of eye affections from opaque eyes **(Fig. 1a)** to congested one

(Fig. 1b), Fish appeared with dark skin **(Fig. 1c),** while The gills appeared congested **(Fig. 1d)**, congested livers were seen in early dead intoxicated fish while paleness of the liver with focal necrotic area and distention of gallbladder were noticed lately **(Fig. 1e)**. Most of these findings were in harmony with those obtained with **Nouh and Selim, (2013)** except of eye lesion which is not referenced before in all exposed checked papers and in partial agreement with those obtained with **Alkobaby and Abd El-Wahed, (2017)** who noticed swelling in liver, gall bladder and pale, damaged gills. gross finding could be explained on a base of direct exposure of these organs to copper as in case of eyes, skin and gills or indirect exposure as in case of liver and kidneys which interfere with process of detoxification and elimination of copper while differences in severity and description of lesions between authors and our present study ,which could be attributed to differences in fish species, immunological status, copper dose and time of exposure. **Group 3 & 4:** Lesions became milder specially in those external organs of the fourth group fish when compared with the third one that could be due to the adsorbent action of sawdust powder in the aquaria which minimize the copper concentration **Ojedokun and Olugbenga, (2015**). On the contrary the internal organs of the third group fish seems with much milder lesions when compared with those of the fourth group which may be due to the direct and indirect antioxidant effect of taurine which minimize toxic effect of copper on these organs which discussed briefly in biochemical part **(Koch *et al.*, 2004)**, while fish of both **groups 5 & 6** showed no gross lesions and tissues looks apparently normal

Histopathological finding:

Group (2)

showed the most pronounced microscopical changes while gills were the most affected part its importance was explained by **Shaw *et al.*, (2012)** who mentioned that gills are a critical organ to fish because of representing the main place for gas exchange, ions regulation, and metabolic waste products excretion. Gills in our study exhibited serious changes represented in severe congestion of blood vessels **Fig. (1)** deformities of the secondary lamellae with or without telangectiasis **Fig. (2)**, which could be explained on a base of direct contact with copper sulphate polluted water severe lymphocytic cells infiltration with fibrosis of soft tissue of gill arch also were noticed in many cases most of these gill lesion was in harmony with those obtained by **Nouh and Selim, (2013) Fig. (3)**. Hepatic lesions represented innumerous areas of coagulative necrosis considered the most common prominent alteration in the hepatic parenchyma intermingled with hepatic vacuolation **Fig. (4)**. Hyperplasia of hemopioetic tissue invests the hepatic blood vessels with or without congestion was noticed **Fig. (5).** Only few cases showed perivascular edema mainly with severe congestion **Fig. (6)**, while diffuse vacuolation of hepatocytes was the marked lesion in case of copper sulphate toxicity **Fig. (7)**, most of these hepatic lesions were in accordance with **Nouh and Selim, (2013) and Alkobaby and Abd El-Wahed, (2017)** who noticed hepatic vacuolation, coagulative necrosis with congestion. Liver affection is very important in copper sulphate toxicity because of its role in the process of detoxification **(Sampaio *et al.*, 2008).** Muscles of the toxicated fish suffered from congested blood vessels and discontinued atrophied muscle fiber **Fig. (8).** Detected copper residues in fish musculature exceeding the permissible limit in our study explained the muscle lesion noticed in this group, this lesion was also agree with that obtained by **Nouh and Selim, (2013)**

Group 3 &4.

Milder changes were detected in both groups unevenly. Gills exhibited mild thickening of primary lamellae in fish of third group **Fig. (9),** while those of fourth group revealed abnormalities in some secondary lamellae **Fig. (10)**. Livers of third group exhibited much milder lesion among all copper sulphate treated groups represented in mild congestion with focal lymphocytic cells infiltrations **Fig. (11)**, while hepatic lesions were more severe in fish of the fourth group when compared with those of the third group and represented in diffuse vacuolation of the hepatocytes with congestion **Fig. (12).** The musculature of the third group fish was less affected than those of fish of the fourth group which exhibited interrupted atrophied fibers **Fig. (13),** while those of the third groups mostly was normal except of mild interstitial edema in some cases **Fig. (14)**. Our microscopical lesions in fish of both **groups 5 & 6** were pararelled with those revealed in gross lesions and showed no alterations and tissues were apparently normal. Modulated lesions in treated groups were explained by **Koch *et al*., (2004), Ojedokun and Olugbenga, (2015) and Wani & Sikdar, (2014).**who noticed the protective role of both taurine and chemically treated sawdust powder in diminishing copper toxicity in ***O. niloticus.***

Conclusion

Our work facing a fact that both models of antitoxic substances were together important as ameliorative agents in copper sulphate intoxicated *O. niloticus* although taurine has superiority in modulation of biochemical results, internal organs pathological alterations beside residues in fish flesh but we can't deny the important role of sawdust powder in modulation the pathological lesion of the external organs which is very important for human during the marketing process**.**

Table (1). Experimental design

Treatment / Group	Copper sulphate 8 ppm	Taurine 5ppm	sawdust powder 0.5 g / 50 ml
Group (1)	--	--	--
Group (2)	+	--	--
Group (3)	+	+	--
Group (4)	+	--	+
Group (5)	--	+	--
Group (6)	--	--	+

Table (2). Prevalence of copper residue in the examined fresh *oreochromisniloticus*samples

Sample	Positive samples		Samples exceeded MPL	
	No.	%	No.	%
Percentage	50	100	7	14

MPL (maximum permissible limit) (20 ppm) according to **(E.O.S.Q.C. 2005).**

Table (3). Copper concentration in examined *Oreochromis niloticus* (n=50).

Values	Min	Max.	Mean ± SE
Cu conc.	1.1	22.4	11.15 ± 0.91

Table (4). Comparison of acceptable daily intake (ADI) values of the detected copper with the calculated daily intake from the examined *Oreochromis niloticus*.

ADI* mg/70kg person	Mean conc. of the copper in the present study (mg/kg)	Calculated daily intake from consumption of 100 gm fish meat daily**	
		mg/day/person	%
35.00	11.15±0.91	1.115	3.18

* ADI: Acceptable Daily Intake of copper according to FAO/WHO, Joint Expert committee on food additives according to **FAO/WHO (1989).** .

****:** The average daily fish consumption for the adult Egyptian person according to **Nutritional Institute, Cairo, Egypt, (1996).**

Table (5). Effect of sawdust powder and taurine on the concentration (mean ± SE) of copper % in fish musculature.

Treatment	Intoxicated group	Sawdust group	Taurine group
Cu conc.	23.30 ± 1.29[a]	17.46 ± 0.78[b]	10.26 ± 0.72[c]
Reduction percent		25.06 %	55.96 %

Means within the same raw carrying different superscripts are significantly different at ($p < 0.05$) based on Duncan's multiple comparisons.

$$\text{Reduction \%} = \frac{(\text{Mean of control} - \text{Mean of treated sample}) \times 100}{\text{Mean of control}}$$

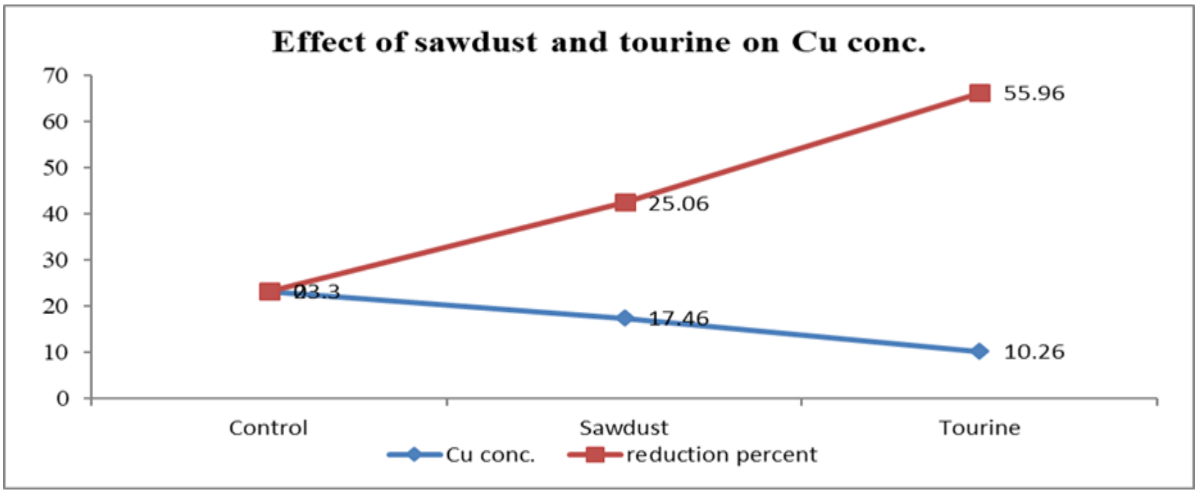

Figure (I). Effect of sawdust and taurine on the concentration (mean ± SE) of copper % in fish muscle

Table (6). The effect of taurine and saw dust (mean± SE) on erythrogram and leukogram of clinically healthy and intoxicated *O. niloticus* with copper sulphate (n =15)

Groups	RBCs (10^6×mm3)	Hb (g/dL)	PCV%	WBCs (10^3×mm3)	Lymphocyte	Neutrophil	Monocyte
Group 1	2.29±0.03 a	8.6±0.088 a	24.4±0.29 a	23.3±0.88 a	62.3±0.88 a	32.4±0.30 a	4.5±0.28 a
Group 2	1.44±0.023 d	6.3±0.057 d	18.9±0.20 c	16±0.57 c	51.3±0.88 c	22.3±0.88 d	2.6±0.33 c
Group 3	2.04±0.023 b	8.1±0.088 b	23.5±0.34 a	22±0.57 ab	59±0.57 b	29.1±0.45 b	3.9±0.20 ab
Group 4	1.9±0.043 c	7.7±0.14 c	22±0.29 b	20.3±0.88 b	57.3±0.45 b	27.1±0.60 c	3.6±0.11 b
Group 5	2.2±0.026 a	8.5±0.057 a	23.8±0.44 a	22.6±0.88 ab	62.6±0.30 a	32.9±0.37 a	4.2±0.23 ab
Group 6	2.21±0.06 a	8.3±0.088 ab	23.5±0.34 a	22±0.65 ab	60±0.85 ab	31.3±0.33 a	4.2±0.14 ab

RBCs: Red blood cells Hb: Hemoglobin PCV%: Packed cell volume WBCs: White blood cells Means with different letters at the same column were significant $P<0.05$

Table (7). The effect of taurine and saw dust (mean±SE) on some antioxidant level and total protein of clinically healthy and intoxicated *O. niloticus* with copper sulphate (n =15)

Groups	GPx (Mg %)	CAT (Mg %)	SOD/ml blood	Total protein(g/dL)
Group 1	1.45±0.026 a	6.9±0.20 a	4.6±0.23 a	4.4±0.11a
Group 2	0.66±0.037 c	4.6±0.23 b	2.6±0.20 b	2.6±0.15c
Group 3	1.42±0.014 ab	6.6±0.17 a	4.6± 0.24 a	3.9±0.088b
Group 4	1.34±0.045 b	6.4±0.23 a	4.3±0.20 a	3.8±0.11b
Group 5	1.52±0.014 a	7.1±0.66 a	5.1±0.57 a	4.4±0.16 a
Group 6	1.45±0.043 a	6.6±0.24 a	4.5 ±0.24 a	4.3±0.088 a

GPx: Glutathione peroxidase activity,CAT: Catalase activity, SOD: Superoxide dismutase activity. Means With different letters at the same column were significant P<0.05

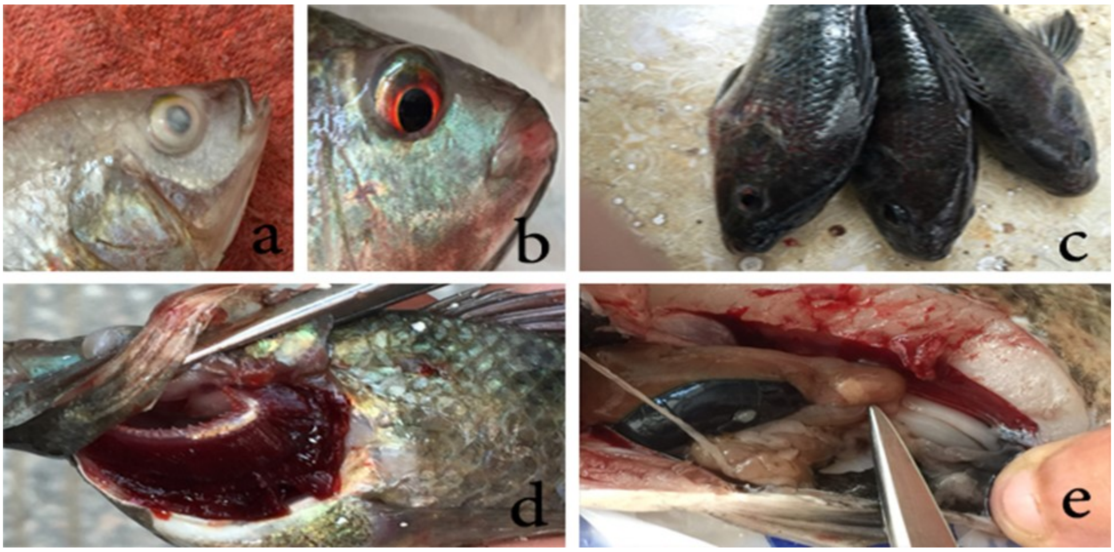

Plate I: Copper sulphate treated fish showing (a): opaque eye at 21days PT **(b)**: other fish appeared with congested eye at **24**th day PT. **(c)**: skin darkness at **30**th day PT **(d):** congested gills with deteriorated ends at **13**thday PT. **(e):** focal whitish necrotic area in pale liver with distended gall bladder

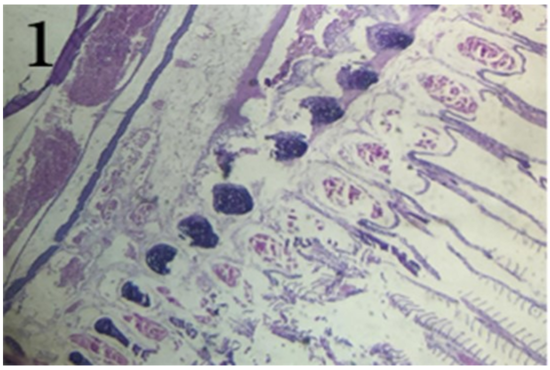

Fig. (1): Photomicrograph of gills of second group fish showing severe congestion **(H&EX200)**

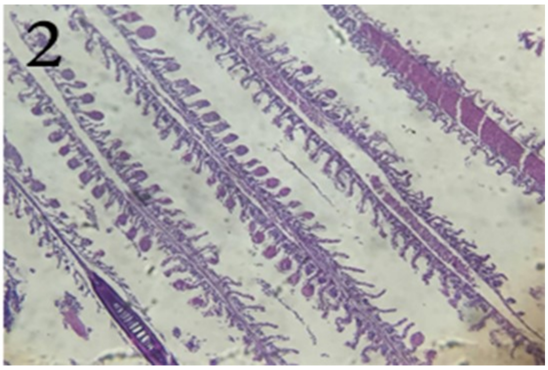

Fig.(2):Photomicrograph of gills of second group fish showing deformities of secondary lamellae with telangectiasis **(H&EX100)**

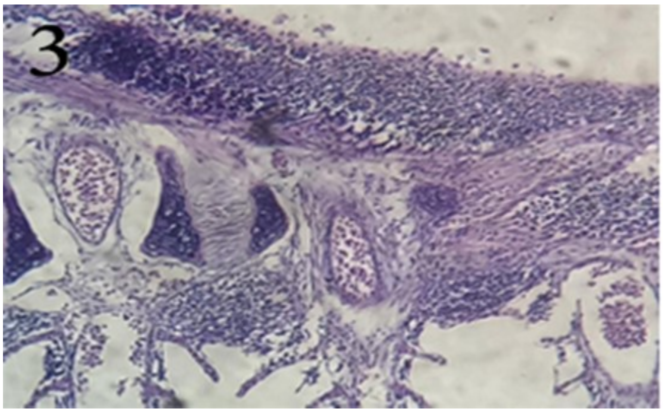

Fig. (3): Photomicrograph of gills of second group fish showing severe lymphocytic cells infiltration with fibrosis of gill arch **(H&EX400)**

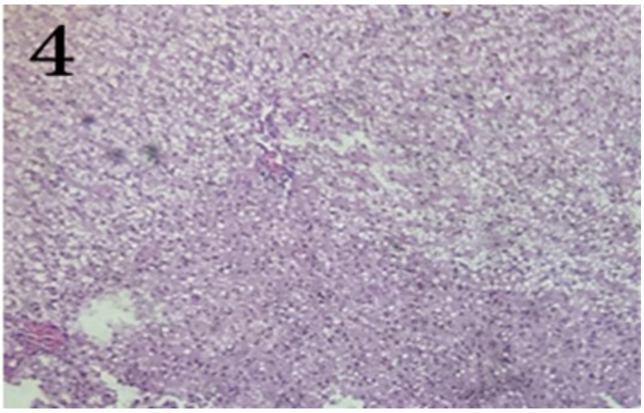

Fig. (4): Photomicrograph of liver of second group fish showing focal area of coagulative necrosis intermingled with hepatic cells vacuolation **(H&EX100)**

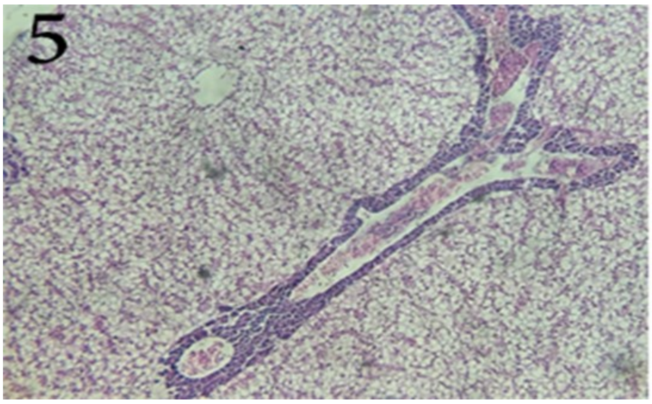

Fig. (5): Photomicrograph of liver of second group fish showing hyperplasia of hemopioetic tissue invests the congested hepatic blood vessels **(H&E X200)**

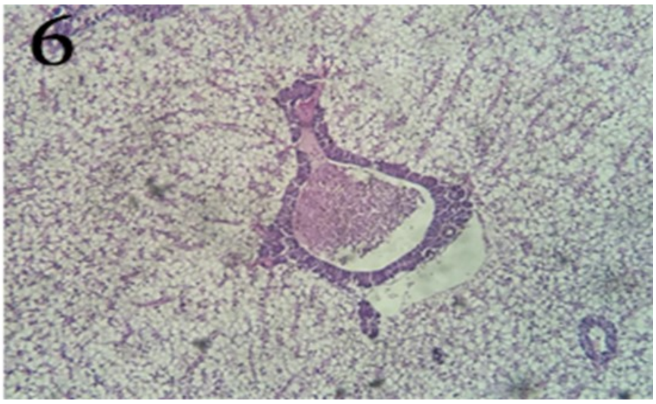

Fig. (6): Photomicrograph of liver of second group fish showing perivascular edema with severe congestion **(H&E X200)**

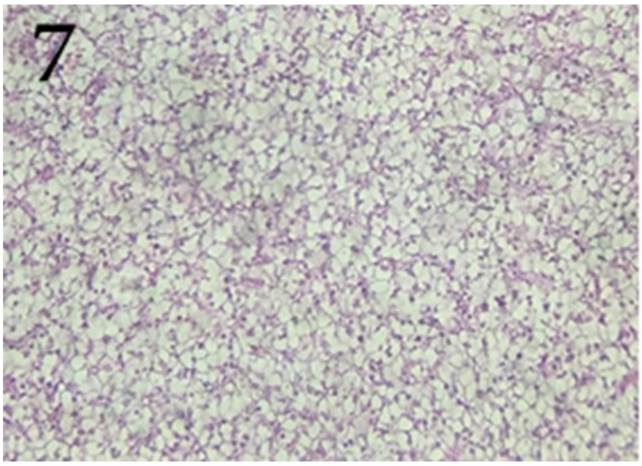

Fig. (7):Photomicrograph of liver of second group fish showing diffuse vacuolation of hepatocytes **(H & E X200).**

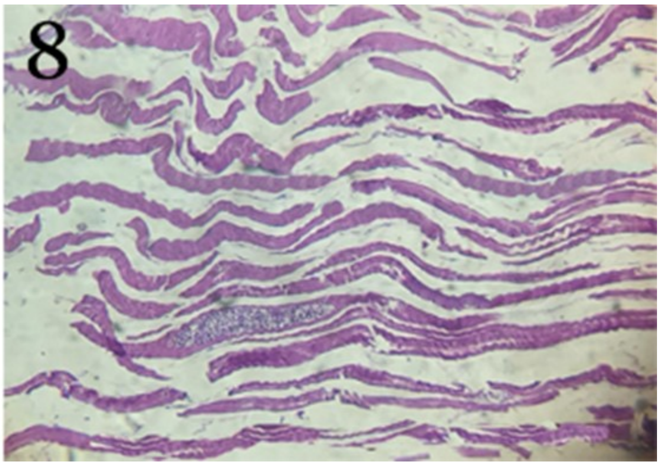

Fig. (8): Photomicrograph of muscle of second group fish showing interstitial edema and congestion of the muscular blood vessels **(H&E X200)**

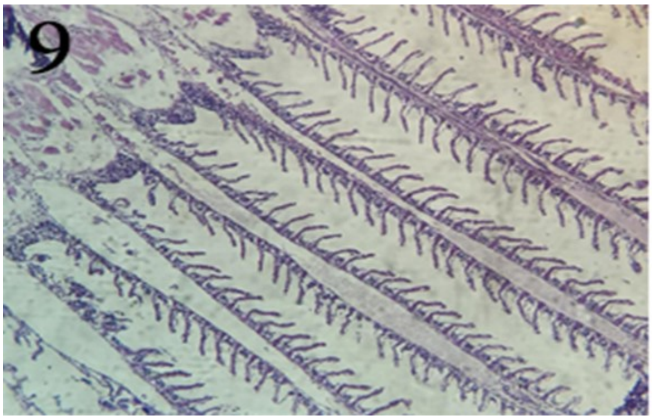

Fig. (9): Photomicrograph of gills of third group fish showing mild thickening of primary lamellae **(H&E X100)**.

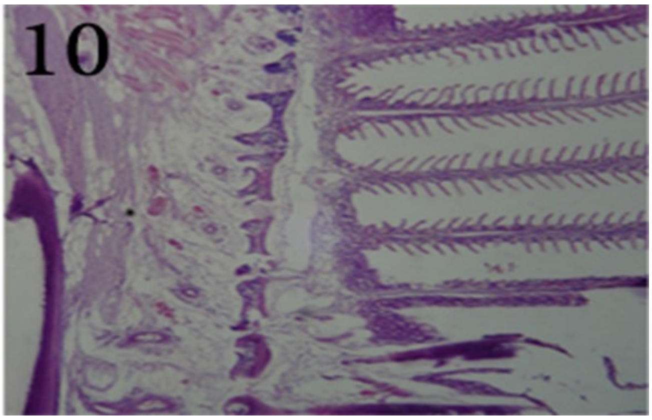

Fig. (10): Photomicrograph of gills of fourth group fish showing abnormalities in some secondary lamellae **(H & EX100).**

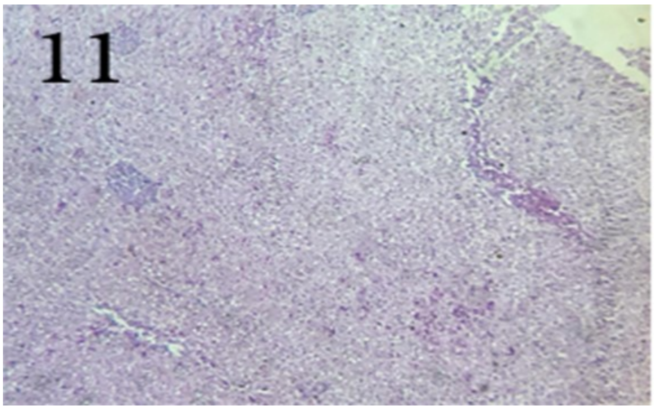

Fig. (11): Photomicrograph of liver of third group fish showing mild congestion of hepatic blood vessels with focal leucocytic cells infiltration **(H&E X 100**)

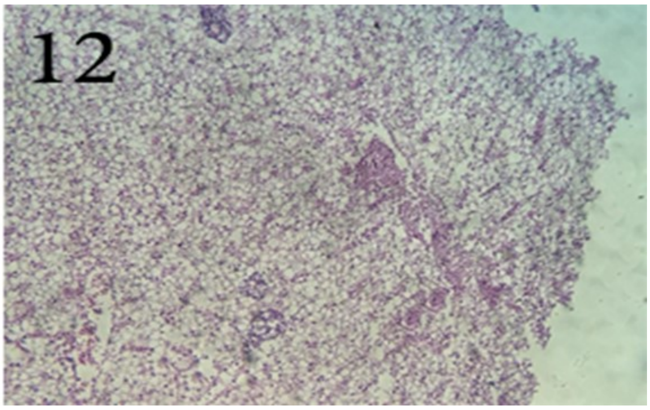

Fig. (12): Photomicrograph of liver of fourth group showing diffuse vacuolation of the hepatocytes with congestion **(H & EX100)**

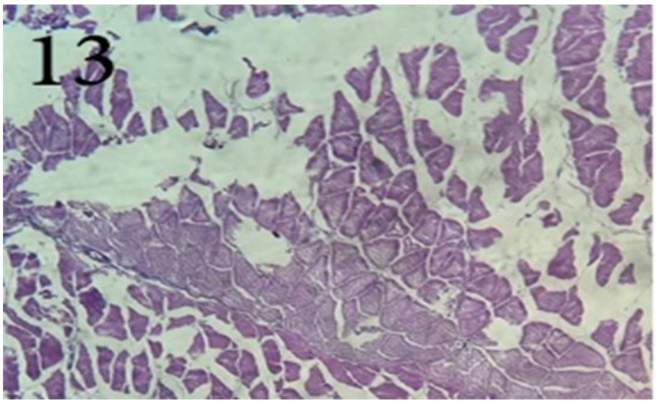

Fig. (13): Photomicrograph of muscle of fourth group showing interrupted atrophied muscle fiber
(H & EX200)

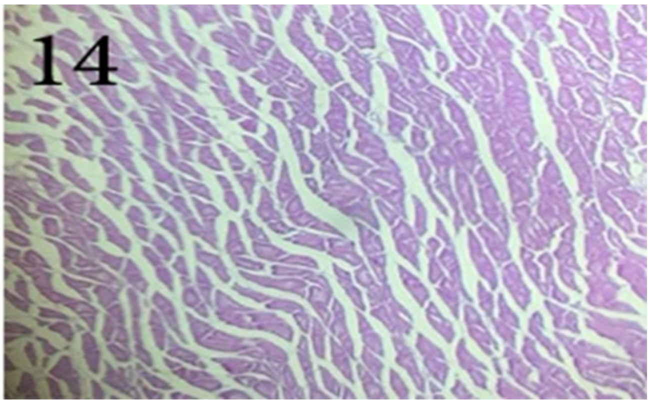

Fig. (14): Photomicrograph of muscle of third group showing mild interstitial edema **(H&EX200)**

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