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Biochemical and Histopathological Effects of Copper Exposure on the Liver of *Oreochromis niloticus* Treated with *Spirulina platensis*

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ABSTRACT

Copper (Cu) is an essential trace metal in aquatic environments. However, excess Cu can induce toxic effects at levels of biochemical and histopathological aspects. Previous studies have reviewed the importance of spirulina supplementation in neutralizing the harmful influences of heavy metals in various fish species. In the present work, the biochemical and histopathological effects of 0.4 and 0.8mg/ l of Cu were addressed on *Oreochromis niloticus* fed spirulina-free diet (SFD) or spirulina-supplemented diet (SSD). Hepatic protein content showed a highly significant increase in fish exposed to 0.8mg/ l of Cu and fed SSD for 7 days. On the 14th day, a significant increase in lipid content was noticed in the liver of fish exposed to 0.8mg/ l of Cu and fed SFD. Both protein and lipid content showed a significant increase in the liver of fish fed SSD without Cu exposure. On the 7th day, a significant increase was found in the liver glycogen of fish exposed to 0.4mg/ l of Cu and fed SFD. While, on day 21, a highly significant increase was found in the liver glycogen concentration in fish exposed to 0.4mg/ l of Cu and fed SSD. Cu induced many pathological lesions including vacuolar degeneration, pyknosis and degenerative and necrotic changes till the loss of architecture and hemorrhagic lesions. The liver histopathological changes observed were more evident with high Cu concentrations and time. The impact of sublethal concentrations of copper on *O. niloticus* is intense. The role of 1% *spirulina platensis* supplementation was limited in neutralizing the harmful influences of Cu on *O. niloticus*

INTRODUCTION

Copper (Cu) is an essential trace metal within aquatic environments and an important micronutrient for many aquatic species (**Zitoun, 2019**). The toxic effects of metals may begin after excretory, metabolic, storage and detoxification mechanisms are no longer able to match the uptake (**Jorgensen, 2012; Kaoud, 2015**). Fish can exhibit serious cellular alteration due to the toxicity impact of Cu **(Al-Bairuty *et al.*, 2013)**. Numerous studies have reviewed the importance of spirulina supplementation in neutralizing the deleterious influences of heavy metals in various fish species **(Bangeppagari *et al.*, 2014; Dar *et al.*, 2014; Vetrivel *et al.*, 2014; Sayed *et al.*, 2017; Mohanty & Samanta, 2018; Priya *et al.*, 2018)**. Moreover, spirulina

supplementation attenuated the toxic effect of some pesticides **(Abdelkhalek *et al.*, 2015; Abdelkhalek *et al.*, 2017; Fadl *et al.*, 2022)**. In the present work, the biochemical and the histopathological effects of 10% and 20% of maximum tolerable concentration (MTC) of copper were examined on *Oreochromis niloticus* fish fed spirulina free diet (SFD) or spirulina supplemented diet (SSD).

MATERIALS AND METHODS

Fish sampling

The present study was carried out on *Oreochromis niloticus* coninciding with the studies of **Trewavas (1981, 1982)**. Fish were reared in NIOF aquarium under healthy conditions. Fish stockage, weighing 9.99 ± 2.6g was done without any feeding during the 24 hour post-transporting. Aeration of aquaria's water was carried out by using air pumps and air stones. The excess food and the fecal matter at the bottom of aquarium was removed and replaced by overnight kept water with the help of plastic tubing using siphon technique. Fish were fed with artificial pellets (30% protein). Two forms of diet were used, spirulina free diet (SFD) and 1% spirulina supplemented diet (SSD). *Spirulina platensis* dried powder was obtained from Hydrobiology lab, NIOF. Cupric sulfate pentahydrate (Lobal Chemei for Laboratory Reagents and Fine Chemicals, India) was used in the present study. Pure compound of cupric sulfate pentahydrate was dissolved in distilled water.

Toxicity screening test

Concentrations of Cu were conducted for toxicity screening in 80-L glass aquaria. Groups of *O. niloticus*, with ten fish for each, were used for toxicity test. One control group was maintained in clean water (control). Other groups were exposed to 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0 and 8.0mg of Cu/ l, respectively. Fish in each aquarium were continuously observed during the period of the experiment (96 hrs). The dead fish were immediately removed upon detection. Mortalities and survival time were recorded.

Chronic exposure to sublethal concentrations of copper in fish fed SFD or SSD

Sublethal concentrations of copper (0.4 and 0.8mg/ l) were used. Two forms of diet were used (SFD and SSD), supplemented with 1% *spirulina platensis* dried powder. The effects of sublethal concentrations of copper were studied after 7, 14, and 21 days via:

Biochemical studies

Samples from the liver of the studied fish were stored in freezer (at −20°C) till used. Samples were subjected for colorimetrical analysis total protein content according to **Gornall *et al.* (1949)** and total lipid content according to **Frings and Dunn (1970)**, using Spectrum Diagnostic kit, Egypt. Meanwhile, glycogen content was measured in fish liver following the anthrone method **(Seifter *et al.*, 1950)**.

Data were collected, coded and entered as spread sheet using Microsoft Excell. Data were analyzed using IBM Statistical package for social science software (SPSS). One way ANOVA test was employed to compare each grouo with control group.

Histopathological studies

The collected samples were fixed in 10% formalin for 24 hours, washed by running water, dehydrated in alcohol, cleared in xylene, and sections of 4-6 µ were prepared according to **Saad *et al* (2012)**.

RESULTS

Toxicity test

The percentage of mortality for *O. niloticus* at different concentrations of copper was recorded. The studied fish tolerated the exposure to 4mg/ l of Cu for 96hrs since no mortality was recorded. Increasing the concentrations of Cu induced the increment of mortality percentage, reaching 100% at 6mg/ l or more of Cu (Table 1).The maximum tolerable concentration (MTC) of Cu for the studied fish was 4mg/ l.

Chronic exposure to sub-lethal concentrations of copper in fish fed SFD or SSD

The effects of Cu (0.4 and 0.8 mg/l) were studied in the liver of the studied fish fed SFD or SSD via:

Biochemical studies

Protein content. On day 7, the liver of fish exposed to 0.8mg/l of Cu and fed SSD induced highly significant increase in the protein content ($P \leq 0.001$) (Table 2). On the 14th and 21st days, no significant differences were detected between control group and other groups (Table 2). Protein content showed significant increase ($P \leq 0.05$) in the liver of fish fed SSD without Cu exposure.

Lipid content. On the 7th and 21st days, no significant difference was observed in the lipid content of the liver of fish exposed to different concentrations of Cu compared to the control. On day 14, a significant increase was detected in the lipid content of the liver of fish exposed to 0.8mg/l Cu and fed SFD ($P \leq 0.05$) (Table 3). Lipid content showed a significant increase ($P \leq 0.05$) in the liver of fish fed SSD without Cu exposure.

Glycogen content. On the 7th day, a significant increase ($P \leq 0.05$) was recorded in the liver glycogen of fish exposed to 0.4mg/ l of Cu and fed SFD.

There were non-significant differences between control and fish exposed to different concentrations of copper on the 14th day. Whereas, on day 21, a highly significant increase ($P \leq 0.001$) was registered in the liver glycogen concentration in fish exposed to 0.4mg/ l of Cu and fed SSD (Table 4).

Table 1. Mortality percentage (%) of *O. niloticus* exposed to different concentrations of copper (Cu)

Copper (mg/l) / Exposure time (hrs)	0 (control)	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0
24 hrs	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**6**	**10**	**10**	**10**
48 hrs	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**
72 hrs	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**
96 hrs	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

n= 10 fish individuals for each group.

Table 2. Comparison between the studied groups as per liver protein concentration (g/100g) of *O. niltoticus* at different follow-up periods.

Cu	Diet	7 days				14 days				21 days			
(mg/l)		Mean	±	SD	P-value	Mean	±	SD	P-value	Mean	±	SD	P-value
0	**SFD**	2.46	±	0.38	-	2.46	±	0.38	-	2.46	±	0.38	-
0	**SSD**	4.40	±	0.59	**0.018***	4.40	±	0.59	**0.018***	4.40	±	0.59	**0.018***
0.4	**SFD**	1.59	±	0.56	0.360	2.48	±	0.84	1.000	3.92	±	1.79	0.911
0.4	**SSD**	1.30	±	0.54	0.105	3.16	±	1.24	0.794	4.60	±	1.56	0.636
0.8	**SFD**	2.54	±	1.38	1.000	3.17	±	0.86	0.785	3.06	±	1.28	0.091
0.8	**SSD**	4.60	±	1.56	**0.001****	6.30	±	4.90	0.875	2.33	±	1.66	1.000

*:p≤0.05 is considered statistically significant **:p≤0.001 is considered statistically highly significant.

SFD: Spirulina Free Diet SSD: Spirulina Supplemented Diet.

Table (3): Comparison between the studied groups as per liver lipids concentration (g/100g) of *O.niloticus* at different follow-up periods.

Cu	Diet	7 days				14 days				21 days			
(mg/l)		Mean	±	SD	P-value	Mean	±	SD	P-value	Mean	±	SD	P-value
0	**SFD**	0.41	±	0.32	-	0.41	±	0.32	-	0.41	±	0.32	-
0	**SSD**	0.92	±	0.24	**0.036***	0.92	±	0.24	**0.036***	0.92	±	0.24	**0.036***
0.4	**SFD**	0.85	±	0.52	0.271	0.62	±	0.46	0.761	0.42	±	0.07	1.000
0.4	**SSD**	0.86	±	0.43	0.236	0.15	±	0.11	0.593	0.24	±	0.13	0.681
0.8	**SFD**	0.49	±	0.22	0.999	0.98	±	0.25	**0.021***	0.44	±	0.18	1.000
0.8	**SSD**	0.36	±	0.23	1.000	0.13	±	0.12	0.508	0.47	±	0.16	0.993

*: $p \leq 0.05$ is considered statistically significant **: $p \leq 0.001$ is considered statistically highly significant

SFD: Spirulina Free Diet SSD: Spirulina Supplemented Diet

Table (4): Comparison between the studied groups as per liver glycogen concentration (g/100g) of *O. niloticus* at different follow-up periods.

Cu (mg/l)	Diet	7 days			14 days			21 days		
		Mean	± SD	P-value	Mean	± SD	P-value	Mean	± SD	P-value
0	**SFD**	2.28	± 0.67	-	2.28	± 0.67	-	2.28	± 0.67	0.99
0	**SSD**	1.82	± 1.25	0.99	1.82	± 1.25	0.99	1.82	± 1.25	0.69
0.4	**SFD**	5.04	± 2.19	**0.01***	1.69	± 1.31	0.94	3.24	± 0.97	0.59
0.4	**SSD**	1.79	± 0.79	0.99	1.86	± 0.96	0.99	3.34	± 0.57	**<0.001****
0.8	**SFD**	2.67	± 1.34	1.00	2.07	± 0.72	1.00	9.72	± 1.81	0.99
0.8	**SSD**	2.34	± 0.66	1.00	2.53	± 1.39	1.00	1.87	± 0.83	0.99

* :p≤0.05 is considered statistically significant ** :p≤0.001 is considered statistically highly significant.

SFD: Spirulina Free Diet. SSD: Spirulina Supplemented Diet.

1.1. Histopathological studies:

As shown in (Fig. 1 A), in the control *O. niloticus*, the liver of fish showed hepatocytes that are arranged in a definite chord-like pattern around sinusoids. The liver of fish fed with SSD without exposure to Cu exhibited pyknosis in the nucleus of some hepatocytes with slight vacuolation in the hepatocytes (Fig. 1 B).

On day 7, *O. niloticus* exposed to 0.4 mg/l Cu and fed SFD showed some pathological lesions in the liver. Vacuolar degeneration of hepatocytes, pyknosis of the nucleus and focal areas of necrosis and, hemorrhage between hepatocytes (Fig. 1 C) was observed. *O. niloticus* exposed to 0.4 mg/l Cu and fed SSD showed moderate recovery of hepatocytes (Fig. 1 D). Meanwhile, infiltration of inflammatory cells and hemorrhage (Fig. 1 D) were noticed.

O. niloticus exposed to 0.4 mg/l copper and fed SFD for 14 days showed increased vacuolar degeneration, increased dilation and congestion of blood sinusoids (Fig. 1 E). In addition, coagulative necrosis (Fig. 1 F) and degenerative and necrotic changes (Fig. 1 G) were presented. *O. niloticus* exposed to 0.4 mg/l copper and fed SSD for 14 days showed a recovery and improvement in many pathologic lesions (Fig. 1 H).

O. niloticus exposed to 0.4 mg/l copper and fed SFD for 21 days showed increased severity of histopathological lesions. The liver showed severe vacuolar degeneration in the hepatocytes and focal areas of necrosis (Fig. 1 I). Coagulative necrosis of hepatocytes (Fig. 1 J) was noticed. Hemorrhage between hepatocytes (Fig. 1 K) was observed. Meanwhile, liver of fish fed SSD and exposed to copper showed less sever lesions. The liver showed vacuolar degeneration in the hepatocytes and thrombosis of hepatic vessel (Fig. 1 L).

On the other hand, the exposure to the higher copper concentration (0.8 mg/l) for 7 days induced more severe vacuolar degeneration of the hepatocytes and pyknosis of the nucleus (Fig. 2 A). However, supplementation of spirulina reduced vacuolar degeneration of the hepatocytes (Fig. 2 B). Moreover, hemorrhagic lesions were observed (Fig. 2 C).

On day 14 after exposure to 0.8 mg/l of copper, the lesions increase in the intensity. The liver showed severe vacuolar degeneration in the hepatocytes (Fig. 2 D), degenerative and necrotic changes and finally loss of the liver architecture (Fig. 2 E). Focal areas of necrosis were observed between the hepatocytes (Fig. 2 F). Hemorrhagic lesions were noticed between the hepatocytes (Fig. 2 G). However, SSD improved hepatocytes appearance with persistent focal areas of necrosis between the hepatocytes (Fig. 2 H) and intravascular hemolysis within hepatoportal blood vessels (Fig. 2 I) were observed.

On day 21 after exposure to 0.8 mg/l of copper, intensive hepatic damage and loss of the liver architecture were found. Severe vacuolar degeneration with hemorrhage between hepatocytes was noticed (Fig. 2 J). Meanwhile, on exposure to 0.8 mg/l of copper and fed SSD, the liver showed severe vacuolar degeneration in the hepatocytes (Fig. 2 K). Also, hemorrhage between hepatocytes (Fig. 2 L) was noticed.

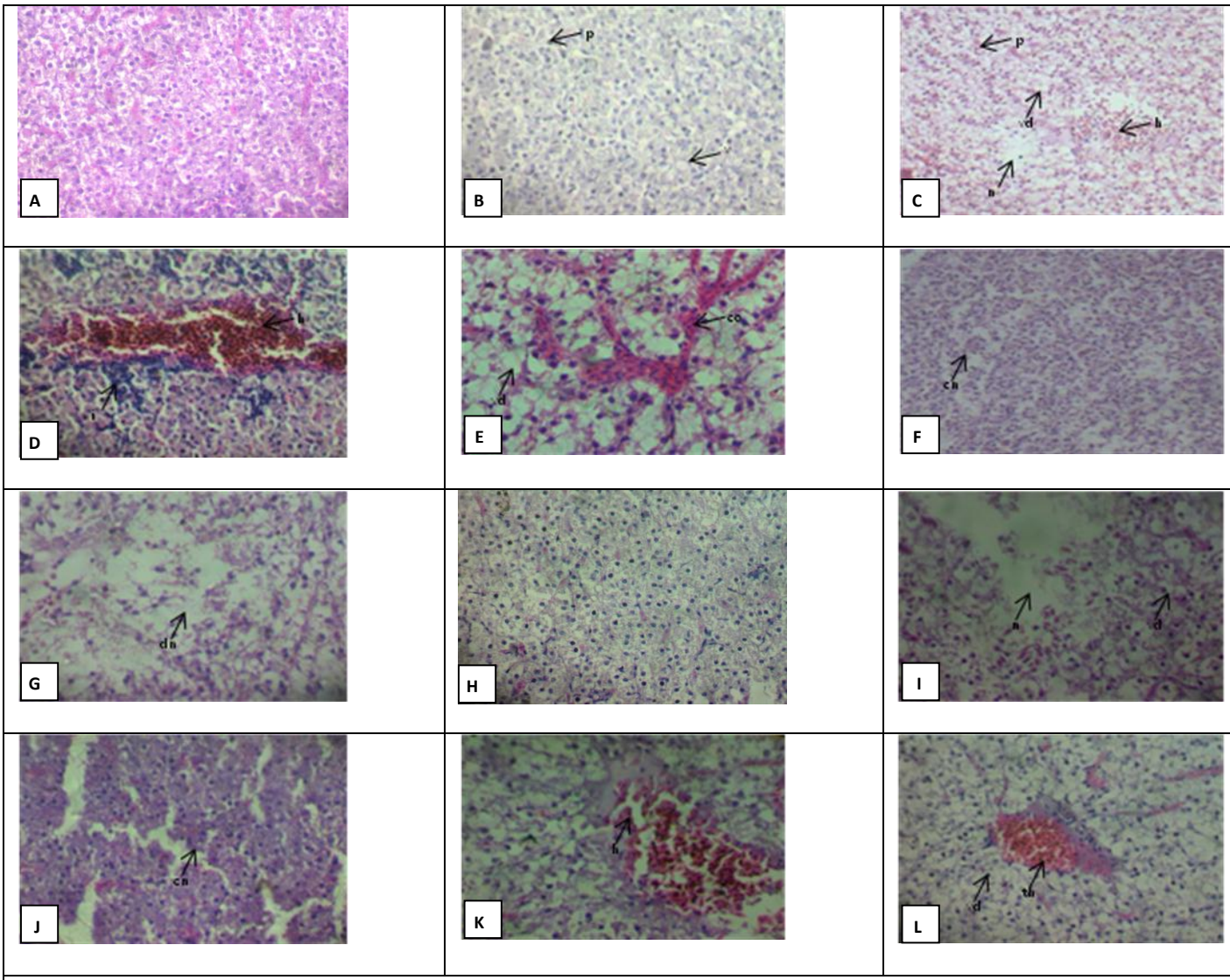

Figure (1): Liver of *o. niloticus* control group fed SFD (A), the liver of fish from control group fed SSD showing pyknosis(p) in the nucleus of some hepatocytes with slight vacuolation(v) in the hepatocytes (B), liver of fish exposed to 0.4 mg/l of Cu and fed SFD for 7 days showing vacuolar degeneration (vd) of hepatocytes, pyknosis(p) of the nucleus and focal area of necrosis(n), hemorrhage(h) between hepatocytes (C), fish exposed to 0.4 mg/l of Cu and fed SSD for 7 days showing moderate recovery of hepatocytes, infiltrations of inflammatory cells(i) and hemorrhage(h) between hepatocytes (D), fish exposed to 0.4 mg/l of Cu and fed SFD for 14 days showing increased vacuolar degeneration(vd), dilation and congestion(co) of blood sinusoids (E), coagulative necrosis(cn) (F) and degenerative and necrotic changes(dn) (G), fish exposed to 0.4 mg/l of Cu and fed SSD for 14 days showing slight recovery of hepatocytes (H), fish exposed to 0.4 mg/l of Cu and fed SFD for 21 days showing severe vacuolar degeneration (vd) in the hepatocytes and focal area of necrosis(n) (I), coagulative necrosis(cn) of hepatocytes (J) and hemorrhage (h) between hepatocytes(K), fish exposed to 0.4 mg/l of Cu and fed SSD for 21 days showing vacuolar degeneration (vd) in the hepatocytes and thrombosis (th) formation in hepatic blood vessel(L))(X400).

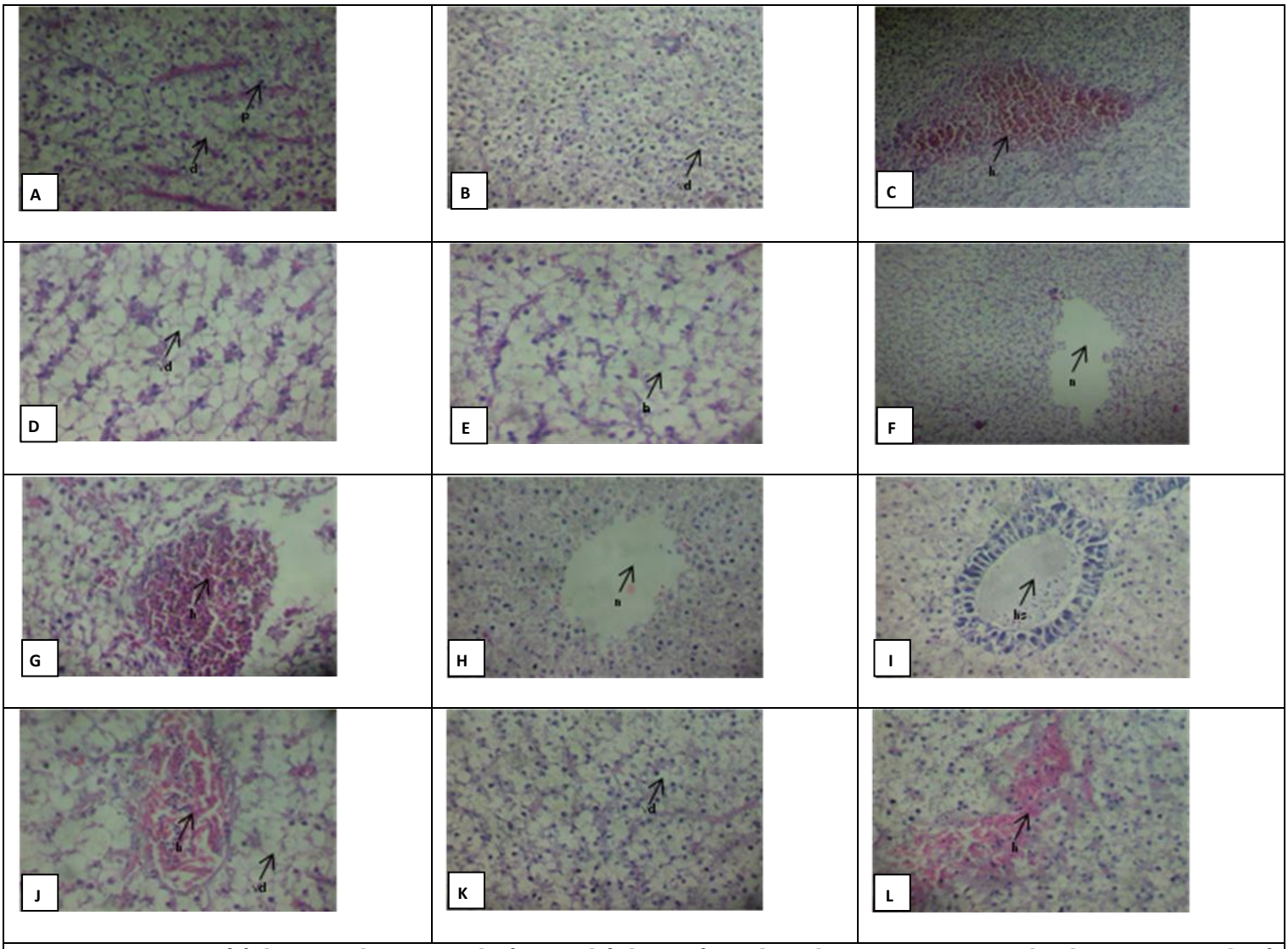

Figure (2): Liver of fish exposed to 0.8 mg/l of Cu and fed SFD for 7 days showing severe vacuolar degeneration (vd) of the hepatocytes, pyknosis (p) of the nucleus (A), fish exposed to 0.8 mg/l of Cu and fed SSD for 7 days showing moderate vacuolar degeneration (vd) of the hepatocytes (B) and hemorrhage(h) between the hepatocytes (C), fish exposed to 0.8 mg/l of Cu and fed SFD for 14 days showing severe vacuolar degeneration (vd) of the hepatocytes (D), loss of the liver architecture(la) (E), focal area of necrosis(n) between the hepatocytes (F) and hemorrhage(h) between the hepatocytes (G), Cu-exposed fish and fed SSD for 14 days showing slight recovery in the hepatic architecture with presence of focal area of necrosis(n) (H) and hemolysis(hs) within hepatoportal blood vessel (I), fish exposed to 0.8 mg/l of Cu and fed SFD for 21 days showing Severe vacuolar degeneration(vd) with hemorrhage(h) between hepatocytes (J), fish exposed to 0.8 mg/l of Cu and fed SSD for 21 days showing severe vacuolar degeneration(vd) (K) and extra hemorrhage (h) between the hepatocytes (L) (X400).

DISCUSSION

There are extensive regulatory requirements for fish acute toxicity data in support of both risk assessment and also hazard classification (**Braunbeck and Lammer, 2006**). This ensures the value of studying sublethal concentrations of different toxins. The maximum tolerable concentration (MTC) is the highest concentration of a substance in an environmental medium that does not cause the death of the test organisms or species. MTC of Cu for *O. niloticus* was 4 mg/l in the present study. Disparate safe, tolerable and toxic levels have been established for Cu on fish. **National Academy of Sciences (NAS)** (1977) reported acute toxicity of Cu at 0.01 – 0.02 mg/L. **Eisler**, (1998) observed that a concentration of Cu between 0.004-0.01 mg/l had sublethal effects to fish and aquatic food chain. Meanwhile, **Bradl, (2005)** reported that Cu concentration is toxic to fish when exceeds 0.02 mg/L. **Mustapha and Agunloye (2016)** found that Cu concentrations ranged from 0.01 to 0.10 mg/l within different aquacultures with the highest concentration recorded in the natural pond and the lowest found in the collapsible pond. The levels of Cu in the ponds were slightly elevated above the references (**Mustapha and Agunloye, 2016**).

`In present work, sublethal concentrations of Cu were selected regarding to MTC. Fish was exposed to 10% and 20% of MTC of Cu (0.4 and 0.8 mg/l, respectively) for 7, 14 and 21 days in presence or absence of spirulina supplemented diet. Complex process of physiological and biochemical changes is involved in fish to cope with stress and nutrient metabolism **(Polymeropoulos *et al.*, 2017)**. In present study, significant increase in protein content in liver of fish fed exposed to 0.8mg/l Cu and fed SSD for 7 days.. The significant increase in hepatic protein content in fish exposed to Cu may be required for fish to meet the increased demand for tissue repair response during Cu exposure **(Javed *et al.*, 2017; Soliman *et al.*, 2021)**. and enhanced by presence of spirulina. Many previous literatures showed the hepatoprotective effects of spirulina against drugs, chemicals and xenobiotics induced hepatic injury **(Abdelkhalek *et al.*, 2015 and 2017).**

The significant raise in the protein content in fish fed SSD without Cu exposure come in accord with previous studies **(Velasquez *et al.*, 2016; Cao *et al.*, 2018; Roohani *et al.*, 2019; Mohammadiazarm, *et al.*, 2021).** This may explained by Spirulina contain high amounts of protein between 55 and 70% by dry weigh **(Zhang *et al.*, 2020).**

Lipids are necessary to maintain cellular energy homeostasis and exert a vast array of functions **(O'Brien *et al.*, 2015).** Pollutants include heavy metals can cause an imbalance between the free radical species production and reduction in fish **(Kamollerd *et al.*, 2019)**. The free radicals can attack lipid, protein and DNA molecules to induce oxidative stress products and DNA damage **(Vilela *et al.*, 2018; Kamollerd *et al.*, 2019; Hashem *et al.*, 2020)**. In present study, significant increase in lipid content in liver of fish exposed to Cu 0.8mg/l and fed SFD for 14 days. Copper exposure induced significant increase in

lipid content in liver of many fishes **(Liu *et al.*, 2010; Chen *et al.*, 2013; Wang *et al.*, 2019).**

Meanwhile, increase of lipid content in liver of studied fish fed SSD without Cu exposure may be as a result of the role of spirulina in increasing concentration of some beneficial long-chain PUFAs and decreased concentration of unwanted SFAs **(Jafari *et al.*, 2014)**.

The glycogen metabolism is the main pathway of energy acquisition, especially in the stressful environment **(Oliveira *et al.*, 2004; Bacca *et al.*, 2005)**. In the present study, the raise of hepatic glycogen content was exhibited in fish exposed to 0.4 mg/l Cu and fed SFD for 7 days and fish exposed to 0.4 mg/l Cu and fed SSD for 21 days. In general, glycogen content reduced during stress because of increased glycogenolysis and decreased glycogen synthesis **(Van Cromphaut, 2009)**. However, metabolic reprogramming may take place to adapt to available energy reserve during stress. Metabolic reprogramming was reported during environmental hypoxia **(Gracey *et al.*, 2011)** for replenishment of glycogen that was over-consumed **(Javed and Usmani, 2015)**. **Li *et al.* (2018)** reported increased glycogen content in chronic hypoxia and decrease in acute hypoxia. Also, the significant increase in glycogen content may be due to enhancement of gluconeogenesis probably due to lactate oxidation through the change of metabolic fuel preference to the use of lactate as a gluconeogenic substrate as occur in chronic hypoxia **(Omlin and Weber, 2010).**

Histopathological study was performed in liver of *O. niloticus* exposed to different concentrations of Cu and/or fed spirulina supplemented diet. The histopathological lesions are highly sensitive and accurate for evaluation of hepatic damages in toxicological studies **(Dabrowska *et al.*, 2012)**. In present study, Cu induced many pathological lesions including vacuolar degeneration, pyknosis, degenerative and necrotic changes till loss of architecture. Hemorrhagic, hemolytic and vascular damages were also observed in liver of fish exposed to Cu. Many literatures agreed with results of present study regarding the alternations induced by Cu such as increase of cytoplasmic vacuolation **(Maharajan *et al.*, 2016; Alkobaby and Abd El-Wahed, 2017; Padrilah *et al.*, 2018)** and nuclear pyknosis **(AL-Bairuty *et al.*, 2013; Maharajan *et al.*, 2016; Sabullah *et al.*, 2017; Alkobaby and Abd El-Wahed, 2017)**.In accordance with literature, Cu induced degenerative-necrotic changes **(Maharajan *et al.*, 2016; Abdel-Tawwab, 2016).** Disorganization and loss of hepatic architecture were also reported after Cu exposure **(Padrilah *et al.*, 2017).** Increased dilation and congestion of sinusoids were reported after Cu exposure **(Padrilah *et al.*, 2017).**

Braunbeck *et al.* (1990) referred that nuclear alterations are signs of increased metabolic activity and pathological action. Pyknosis is one of hepatocellular pleomorphism thought to be exhibited in sub lethally injured cells resulting from exposure to several types of toxicant **(Agamy, 2012).** Cu toxicity increase ROS formation and decrease antioxidant protection causing oxidative stress. This may result in structural

changes in biomembranes, loss of liver integrity, decreased metabolic activity, and finally cell death **(Daggulli *et al.*, 2014)**. Increased cytoplasmic vacuolation might indicate imbalance between the synthesis rate of different molecules in parenchymal cells and the rate of releasing them into systemic circulation **(Hadi and Ahwan, 2012)**. Meanwhile, increased vacuolation of the hepatocytes is a degenerative process suggestive of metabolic damage **(Pacheco and Santos, 2002)**. Degenerative necrotic conditions could be explained by Cu-induced oxidative stress in the tissue of the liver **(Holye *et al.*, 2007)**. Necrosis of some portions of the liver tissue is attributed to presence of chemicals disturbing the physiological process within cells **(Mela *et al.*, 2007)**. Also, hepatic degeneration and necrosis may be resulted from stasis of the blood and impaired blood flow due to the vascular engorgement and congestion of the blood vessels and sinusoids **(Mohamed, 2001).** Loss of the hepatic architecture is probably accompanied with cell necrosis and degeneration of structural proteins in the membrane of the hepatocytes **(Pacheco and Santos, 2002)**.

The degenerative alterations and necrosis are given the highest importance factor because they are considered a direct effect of toxicants, they are generally irreversible, and their persistence or progression may lead to partial or total loss of organ function **(Agamy, 2012; Biuki *et al.*, 2013; Mela *et al.*, 2013; Abdel-Moneim, 2014; Afifi *et al.*, 2014).**

Hemolysis and hemorrhage are a result of blood channel disruption, and indication of severe physical damage. The hemolysis, dilation, and congestion may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals.

Infiltration of inflammatory cells was seen in liver of fish exposed to Cu and fed SSD. **Kevat *et al.* (1988)** owed cellular infiltration to inflammation and reaction of microcirculation and movement of fluids and leukocytes from the blood into the extra vascular tissue. This may be line of immunological response. Also, that was classified by other researchers as a moderate injury and it is often associated with neutralization and destroying the source aggressor, cleaning the tissue by removing dead cells, as well as inducing the recovery of damaged tissue **(Bernet *et al.*, 1999)**.

The liver histological changes observed were more evident in fish exposed to the higher Cu concentrations in agreement with **Al-Tamimi *et al.* (2015)** and **Padrilah *et al.* (2017)**. Also, the degree of liver histopathological lesions induced by Cu seemed to be more pronounced with time. These histological lesions were considered indicative of the impact of Cu on the health of *O.niloticus* fish.

In present study, spirulina supplementation induced moderate recovery in hepatocyte morphology. **Soliman *et al.* (2021)** found that exposed fish pre-fed the basal diet supplemented with spirulina maintained a more normal histological structure than those that were exposed to $CuSO_4$ without supplementation. Meanwhile, many pathological lesions were still well presented in Cu exposed fish liver during feeding SSD. This may

be owed to contamination with cyanotoxins or toxic metals **(Kerna *et al.*, 2022)**. Meanwhile, spirulina reported to produce toxins (microcystins, β-methylamino-L-alanine (BMAA) which might contribute to liver damage **(Simran *et al.*, 2022)**.

CONCLUSION

In current study, the impact of sublethal concentrations of copper on *o. niloticus* was intense. The role of 1% spirulina supplementation in neutralizing the harmful influences of Cu on *o. niloticus* was limited.

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