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PROTECTIVE ROLE OF SPIRULINA ON HEMATOLOGY AND HEPATOTOXICITY INDUCED BY $CUSO_4$ AND CUO NANOPARTICLES IN *OREOCHROMIS NILOTICUS*

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ABSTRACT

Copper toxicity poses significant risks to fish. *Spirulina platensis* a cyanobacterium (protist), has shown protective properties against metal-induced toxicity in various species. This study investigated the prophylactic role of *Spirulina platensis* in Nile tilapia (*Oreochromis niloticus*) against the toxicity of copper sulfate ($CuSO_4$) and Copper oxide nanoparticles (CuO-NPs). Over 15 days, tilapia were divided into five groups: For this purpose, fish were divided into five treatments. T_0 was a controlled treatment. T_1 was treated with $15mgl^{-1}$ CuO-NPs. T_2 was fed with $2.5gkg^{-1}$ Spirulina and $15mgl^{-1}$ CuO-NPs. T_3 was treated with $15mgl^{-1}$ $CuSO_4$. T_4 was treated with $2.5gkg^{-1}$ Spirulina and exposed to $15mgl^{-1}$ $CuSO_4$. $CuSO_4$ and CuO-NPs were given for the last 15 days and Spirulina was given on the first day of the trial in T_2 and T_4. The results showed that both $CuSO_4$ and CuO-NPs significantly increased biochemical parameters like total ALP, ALT and AST. Hematological disturbances and erythrocyte count along with histopathological damage to organs like the liver were also observed. However, Spirulina supplementation notably mitigated these adverse effects, reducing and preserving tissue integrity and normalizing all parameters. This study highlights the species-specific protective effects of the agent and suggests that Spirulina may offer protection against copper-induced toxicity. Future research is needed to explore the long-term impacts and underlying mechanisms of these agents, along with their optimal dosages for enhancing fish health in aquaculture.

Keywords:

Copper oxide nanoparticles, Copper sulphate, Hepatotoxicity, Haematological indices, biochemical parameters, *Spirulina platensis*, *Oreochromis niloticus*,

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1. Introduction:

Copper is a crucial micro and nanoscale bioelement. It powers many metabolic activities but it is toxic to aquatic organisms. It also shows harmful effects to human cellular activities [1]. Nanoparticles also used in paint, plastic and textile industries due to their biocidal characteristics. Copper and copper nanoparticles are now in many industries. They are used in electronics, construction, and biocides [2]. Copper sulphate are applied to degrade toxic algal and fungal blooms in aquaculture [3]. The overusing of copper oxide nanoparticles harms ecosystems and aquatic life [2]. Toxic effects derived by these practices are increasing fish mortality rate at enormous level and the cause decrease in economics contributions [4]. Nanoparticles are drived from natural resources and their production is anticipated to increase 500,000 tons by 2020 [5]. Traditional extraction method to synthesized CuO nanoparticles including precipitation [6, 7], sol-gel [8-10] and electrochemical processes [11-13]. These nanoparticles enter into the aquatic ecosystem through industrial effluents, surface runoff, sewage and airborne accumulation relating with indigenous species and cause the cellular toxicity [14].

Toxic level of these metal and metal oxide nanoparticles may effects different body organs e.g. liver and blood cells with changes examined by biochemical, histological and histopathological studies [15]. Heavy metals adversely affect the fish physiology, growth and reproduction influencing sustainable aquaculture [16]. Toxic pollutants change fish behaviours, feeding way and social interaction which leads to overall disturbance in hormonal changes and sensory impairments [16]. Different studies examine the impact of heavy metals and particle on fish behavioural biochemical parameters, antioxidant unlimited activities and histopathological changes[17].

The cyanobacterium *Spirulina platensis* has reported protection against metals-based toxicity in different aquatic species [18, 19]. Spirulina carry high significant amount of biomolecules like amino acids, fatty acids, proteins, vitamins and minerals [20, 21]. *Spirulina platensis* also have additional bioactive compounds with prominent properties like antioxidant e.g. phycocyanin and allophycocyanin [22, 23]. In fish aquaculture, Spirulina contributions recently as a source of important food supplements as well as toxins treatment of aquatic waste water [21]. However, the use of microalgae or plant extract as bioactive compounds to reduce CuO-NPs toxicity [24]. Particularly, Spirulina extract proffer preservatives role against $CuSO_4$ toxicity in fisheries and other aquatic organisms. Therefore, toxicity of $CuSO_4$ and CuO-NP as well as potential defensive character of Spirulina against such toxins [25-27]. Nile tilapia (*Oreochromis niloticus*) is considered as one of the most precious freshwater fish in Egypt and Africa. This specie reproduction rate and various effluents tolerability make it excellent model for toxicological studies [28]. The present study emphasis on detrimental effects of $CuSO_4$ and CuO-NPs and their possible protective effects of Spirulina supplements were examined in Niles tilapia. Changes were showed in different parameters like biochemical parameters, histopathological changes and haematological indices.

2. Materials and Methods

2.1. Ethical Statement:

All experiments were carried out following the rules and regulations adopted by the ethical committee of Agriculture University Faisalabad, Pakistan.

2.2. Experimental Site and fish collection:

CuO-NPs were prepared in laboratory of Chemistry department of University of Agriculture Faisalabad. The stock solution of CuO-NPs was prepared according to the method Soliman, Hamed and Sayed [29]. The properties of the nanoparticles were examined through FTIR, UV-Visible spectrometer and zeta potentiometer. $CuSO_4$ particularly ($CuSO_4.5H_2O$) were applied as a source of Copper ion. Spirulina and green tea extract were prepared in the high-tech central lab of Pakistan Agriculture Research Station (PARS) at University of Agriculture Faisalabad. Early juveniles of Nile tilapia were taken from Tawakkal Fish Hatchery & Farm Muzaffargarh (Punjab, Pakistan) and transported to the Laboratory of Department of Zoology, Wildlife and Fisheries Biology at University of Agriculture Faisalabad. The fish were checked to make sure that they are free from any external toxic agent like parasitic body and are healthy.

2.3. Acclimatization of fish:

The fingerlings (n=180) Tilapia of both sexes were evenly distributed into rectangular glass tanks (170 x 90 x 60 cm) each containing 460 liters of dechlorinated water. Fish were acclimatize to laboratory environment for 7 days and fed with commercial diet. To guarantee proper oxygen saturation the tanks were aerated using air stones connected to an air compressor.

2.4. Experimental setup:

The water's physiochemical properties were assessed and the fish were divided into six treatments each consisting of 30 fish:

Control Group: Fish pre-fed a normal diet.

CuO-NPs Group: Fish fed a normal diet and exposed to 15 mgl^{-1} of CuO-NPs.

CuO-NPs + Spirulina Group: Fish fed a diet supplemented with 0.25% Spirulina and exposed to 15 mgl^{-1} of CuO-NPs.

$CuSO_4$ Group: Fish fed a normal diet and exposed to 15 mgl^{-1} of $CuSO_4$.

$CuSO_4$ + Spirulina Group: Fish fed a diet supplemented with 0.25% Spirulina and exposed to 15 mgl^{-1} of $CuSO_4$.

Each aquarium received a daily diet equivalent to 4% of the total weight of fish within it. The fingerlings were fed twice a day once from 08:00-09:30 am and again from 03:00-04:00 pm. To account for changes in body weight 5% of the fish were randomly sampled after the trails. After giving feeding all fecal matter was siphoned off before the next feeding. After feeding, unconsumed feed was removed by pipetting out.

Table 1: Experimental Design for the Assessment of Copper Toxicity and Spirulina Supplementation in Nile Tilapia (*Oreochromis niloticus*)

Treatment	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**Duration**
Diet	Commercial diet	Commercial diet	Pre-fed with commercial diet+ with 0.25% spirulina	Commercial diet	Pre-fed with commercial diet+ with 0.25% spirulina	For 3 months
Treatment	No exposure	CuO nanoparticle 15mgl^{-1}	CuO nanoparticle 15mgl^{-1}	$CuSO_4$ 15mgl^{-1}	$CuSO_4$ 15mgl^{-1}	For 21 days

The experimental design implemented to investigate the effects of copper oxide nanoparticles (CuO-NPs) and copper sulphate ($CuSO_4$) on Nile tilapia, with or without spirulina supplementation, is presented in the table. A commercial food was given to five groups, with the T_2 and T_4 groups receiving pre-fed spirulina (2.5 gkg^{-1}). After three months feeding period, groups (T_0, the control group) were exposed to 15 mgl^{-1} of CuO-NPs (T_1 and T_2) or $CuSO_4$ (T_3 and T_4) for 21 days.

2.5. Processing of Spirulina:

Select all the appropriate feed ingredients for processing. Weigh raw ingredients for accurate amount of different nutrients. Grind the mixture to obtain homogenous mixture of specific particle size to improve digestibility, palatability and water stability of the feed. Mix the ingredients with hot water and add binders except vitamin mixture as it is heat sensitive. Knead the dough well. The dough is then wrapped in silver foil and autoclaved for 20 min at 110-120°C temperature to make it free from microorganisms.Addition of vitamin and minerals. After autoclaving, the feed mixture is allowed to after which the pre-weighed vitamin and mineral mixture is added and mixed well. In this process the prepared feed mix is allowed to pass through the extruder for preparing the feed pellets. The pellets are appropriately dried as per the requirements. After drying the final feed product can be packed in HDPE Bags. Store the feed bags in a clean place that is free from pests and insects. Air circulation must be maintained. Bags of feed should be printed with date of manufacturing.

Preparation of Feed for Nile tilapia with spirulina

Table 2: Formulation of composition of the experimental diets or Fish feed ingredients for *Oreochromis niloticus*.

Ingredients	**T0**	**T1**	**T2**	**T3**	**T4**
Spirulina	0	0	2.5	0	2.5
Corn	2	2	2	2	2
Rise Polish	15	15	12.5	12.5	12.5
Rapeseed meal	0.5	0.5	0.5	0.5	0.5
Canola gluten	30	30	30	30	30
Poultry meal	45	45	45	45	45
Fish meal	1	1	1	1	1
DCP	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5
Methionine	1	1	1	1	1
Minerals	1	1	1	1	1
Vitamins	1	1	1	1	1
Molasses	0.5	0.5	0.5	0.5	0.5
Synthetic amino acids	2	2	2	2	2
Total	100	100	100	100	100

2.6. Blood Analysis

The lab work setup, guidance and fish handling process were accepted by the Research and ethical committee of the faculty affairs University of agriculture Faisalabad. Measurement of biochemical antioxidant were taken and observed using a spectrophotometer with slight modifications. Further hematological parameters were evaluated through complete blood count test. Blood sample along with serum were collected from caudal vein to evaluate biochemical parameters, antioxidant biomarkers and hematological indices. Moreover, complete juvenile was utilized for histopathological studies. They were given 25 mgl^{-1} of clove oil to induce unconsciousness. After drawing blood with a sterile 1 milliliter syringe from the caudal vein, the samples will be promptly moved to an EDTA coated vial which acts as an anticoagulant and be stored at 4°C until further examination. Hematocrit (Hct) were determined by using the hematocrit method.

Red Blood Cells:

Total no of red blood cells found at per cubic millimeter was measured as

$$\text{Total RBCs count} = \frac{\text{n Dilutionfactor}}{\text{Area Depth}}$$

n = Number of red blood cells counted in squares

Dilution factor = 1/100

Area = area of 80 squares 1/50 sq.mm

Platelets:

$$\text{Total platelets count} = \frac{\text{n Dilution factor}}{\text{Area Depth}}$$

N= number of platelets counted

Area = Area of 5 squares 1/5 sq.mm

Depth of chamber = 0.1 mm

Dilution factor = 20

Haemeoglobin:

Hb (g/dl) = Absorbance of test sample absorbance of standard x concentration of standard

Dilution Factor 1000

Hematocrit

Hematocrit value was attained on the hematocrit reader value was calculated by following equation

Height of packed Red cells × 100 Hematocrit × Height of packed Red cells and Plasma

Mean Corpuscular value

$$MCV = PCV \times 10 \text{ RBC Count}$$

RBC Count = Red blood cell count

MCV = Mean Corpuscular value

PCV = Packed Cell Volume

2.7. Measurement of biochemical level:

ALT, AST and ALP are liver functioning enzymes and are used as serological makers for determination of liver injury. Blood samples were collected in gel containing tubes and centrifuged at 3000 rpm for the separation of serum. The serum was stored at -20°C tempera-ture for biochemical analysis. Resulting serum was analyzed for the determination of alanine aminotransferase (ALT), alkaline phosphate (ALP) and separate aminotransferase (AST). Tests for biochemical analysis were performed at the Clinical Laboratory of University of Agriculture Faisalabad. Cortisol level were analyzed using an electrochemiluminescence immunoassay on a Cobas analyzer.

Following equation is used for the determination of activity of ALP:

Chemical Equation

P-nitrophenylphosphate+H_2O→ P-nitrophenol yellow + Phosphate

Following equation is used for the determination of activity of ALT:

$$Activity\ of\ ALT = \frac{B \times Sample\ dilutionfactor}{T \times V}$$

The number of pyruvates produced between Tinitial and Tfinal(in mole)= B

Reaction Time= T= Tfinal-Tinitial(min)

Volume of sample (ml) poured to the well=V

Following equation is used for the determination of activity of

$$Activity\ of\ AST = \frac{B \times sample\ diututionfactor}{T \times V}$$

The number of glutamates produced between Tiniitial and Tfinal (in mole) = B

Reaction Time-T= Tfinal-TInitial (min)

Volume of sample (ml.) poured to the well=V

2.8. Histopathological analysis:

Livers were extracted from all treatments. All the liver samples were placed into bottles and were immediately fixed in 10% neutral buffered formalin and processed daily before being stained with Harris' hematoxylin and eosin (H&E). Tissue section were made from microtome and then examined with the microscope to determine the abnormalities of tissues by microscopic studies. Histology performed of liver tissues was to determine the extent of Copper toxicity and the beneficial effects of Spirulina.

2.9. Statistical analysis:

To determine the differences between the means of each experimental group, one-way ANOVA was used to analyze the data in the Excel sheets, and Tukey's post-hoc test was then performed. $P < 0.05$ was the threshold for statistical significance. Version 22 of SPSS was used for all analyses.

3. Results

3.1. Characterization of CuO-NPs

For determining absorption end and band gap energy, SP65 spectrophotometer scanning calculated by UV-visible disseminated biosynthesized photocatalysts up to 200nm to 800nm.

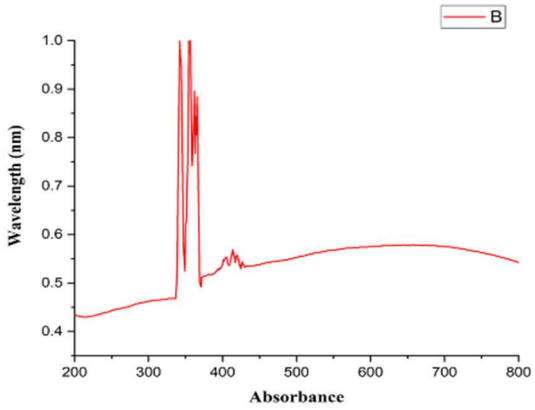

Figure 1: Characterization of Copper oxide nanoparticle through UV-Visible Spectrophotometer

CuO nanoparticles hexagonal wurtzite structure analyzed by XRD technique analysis at different levels of 10°, 12°, 15°, 18°, and 20°, and optimum particles measured 56.72 nm by Debye-Scherrer's formula.

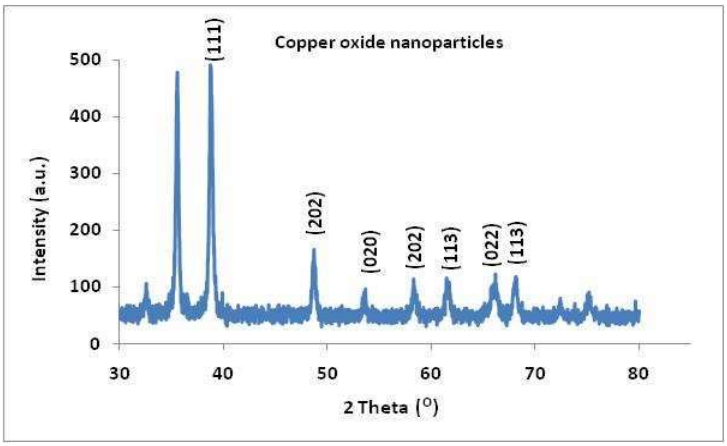

Figure 2: Characterization of Copper oxide nanoparticle through XRD technique

The characterization of CuO nanoparticles by FTIR spectroscopy showed vibration at 432, 511, and 610 cm^{-1} with vibrational modes of water molecules at 886, 1630 and 3398 cm^{-1} and a triply degenerate ν_3 mode of SO_4^{2-} ions.

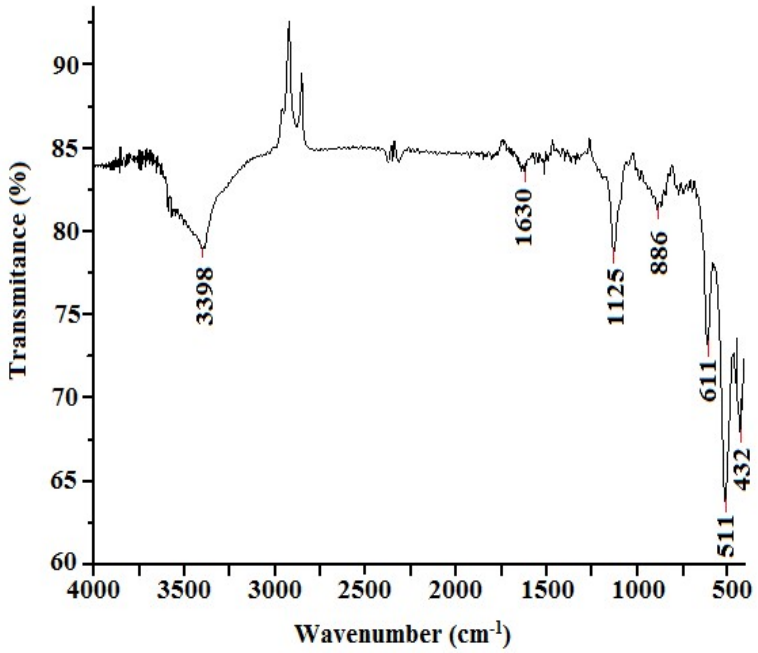

Figure 3: Characterization of Copper oxide nanoparticle through XRD technique

3.2. Biochemical parameters

Fish were exposed to $CuSO_4$ and CuO-NPs their alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were significantly higher ($P < 0.05$) than in the control group (Table 3). Furthermore, compared to the control group, blood levels of aspartate aminotransferase (AST) increased significantly ($P < 0.05$) after exposure to $CuSO_4$. Overall, it was shown that CuO-NPs were less toxic than $CuSO_4$. On the other hand, fish that were pre-fed with spirulina, all of the Previously mentioned biochemical parameters were within the normal range. This suggests that spirulina assisted in reducing the biochemical effects of the copper compounds.

Table 3: Effects of exposure to copper oxide nanoparticles or copper sulphate for 21 days on the Biochemical parameters of early juvenile Nile tilapia (*Oreochromis niloticus*) fed a basal diet with or without supplemental *Spirulina platensis*

Parameters	Treatment 0	Treatment 1	Treatment 2	Treatment 3	Treatment 4
	Commercial diet	15mgl^{-1} CuO-NPs	15mgl^{-1} CuO-NP +2.5gkg^{-1} Spirulina	15mgl^{-1} $CuSO_4$	15mgl^{-1} $CuSO_4$ +2.5gkg^{-1} Spirulina
Biochemical parameters					

AST	55.3±4.1ab	55±2ab	55.3±2.0ab	60±2.6a	55.3±0.7ab
ALT	29.6±0.52b	30±0.2ab	29.8±0.30b	31.4±0.7a	30.46±0.8ab
ALP	32.4±1.3b	33.2±1.1ab	32.7±0.6b	34.2±0.5a	33.6±0.5ab

The means ± SEs of the data are displayed. Significant differences ($P < 0.05$) are indicated by values with different superscript letters within the same row for each parameter. Group 0 was the control group, fed the baseline diet and kept in untreated water. After receiving the standard diet, Group 1 was given 15 mgl^{-1} of copper oxide nanoparticles. After receiving the base diet with 0.25% spirulina, Group 2 was given 15 mgl^{-1} of copper oxide nanoparticles. After receiving the standard meal, Group 3 was given 15 mgl^{-1} of copper sulphate. After receiving the base diet plus 0.25% spirulina, Group 4 was exposed to 15 mgl^{-1} of copper sulphate.

3.3. Hematological parameters

Hematological parameters such as platelet counts, blood hemoglobin concentration, hematocrit and erythrocyte count (RBC) significantly ($P < 0.05$) decreased after being exposed to $CuSO_4$ and CuO-NPs in contrast to the findings from the control groups (Table 4). Only after being exposed to $CuSO_4$ did the mean corpuscular volume exhibit a substantial reduction ($P < 0.05$) (Table 4). On the other hand, following exposure to $CuSO_4$, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and total white blood cell count were all considerably greater ($P < 0.05$) than in the control group (Table 4). It was discovered that CuO-NPs were less hemotoxic than $CuSO_4$. Moreover, the majority of the alterations in haematological parameters brought on by $CuSO_4$ and CuO-NPs were lessened by pretreatment with spirulina.

Table 4: Effects of exposure to copper oxide nanoparticles or copper sulphate for 21 days on the Haematological parameters of early juvenile Nile tilapia (*Oreochromis niloticus*) fed a basal diet with or without supplemental *Spirulina platensis*

Parameters	**Treatment 0**	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**
	Commercial diet	**15mgl^{-1} CuO-NPs**	**15mgl^{-1} CuO-NPs +2.5gkg^{-1} Spirulina**	**15mgl^{-1} $CuSO_4$**	**15mgl^{-1} $CuSO_4$ +2.5gkg^{-1} Spirulina**
Hematological parameters					

RBCs	1.92±0.01a	1.83±0.02b	1.86±0.008 ab	1.78±0.03b	1.84±0.01 b
Haemoglobin	9.36±0.16a	8.6±0.2b	8.8±0.02ab	8.56±0.16b	8.7±0.07a b
WBCs	11.5±0.1a	9.26±0.4b	8.96±0.2b	14.5±0.3c	12.2±0.3ac
Platelets	22.63±0.2a	20.23±0.1b	21.36±0.4a b	20.53±0.3b	21.53±0.3 ab
Haematocrit	26.5±0.3a	25.13±0.3b	26.26±0.3a b	24.36±0.4c	25.96±0.1 ab
MCV	137.3±1.5a	134±1b	138±2ab	131.6±1.5c	136±2ab

The means ± SEs of the data are displayed. Significant differences ($P < 0.05$) are indicated by values with different superscript letters within the same row for each parameter. Group 0 was the control group, fed the baseline diet and kept in untreated water. After receiving the standard diet, Group 1 was given 15 mgl^{-1} of copper oxide nanoparticles. After receiving the base diet with 0.25% spirulina, Group 2 was given 15 mgl^{-1} of copper oxide nanoparticles. After receiving the standard meal, Group 3 was given 15 mgl^{-1} of copper sulphate. After receiving the base diet plus 0.25% spirulina, Group 4 was exposed to 15 mgl^{-1} of copper sulphate.

3.4. Liver histopathology

The hepatocytes in the control fish were arranged around the central vein in a cord-like pattern, with blood sinusoids strewn throughout (Fig. 4a). On the other hand, the livers of fish exposed to CuO nanoparticles (CuO-NP) as well as $CuSO_4$ showed histological alterations, such as nuclear pyknosis and hepatocyte vacuolization (Fig. 4b, d). In contrast to the fish exposed to the treatments without the supplementation, the fish that were pre-fed a basal diet enriched with spirulina displayed a more conserved histological structure (Fig. 4c, e).

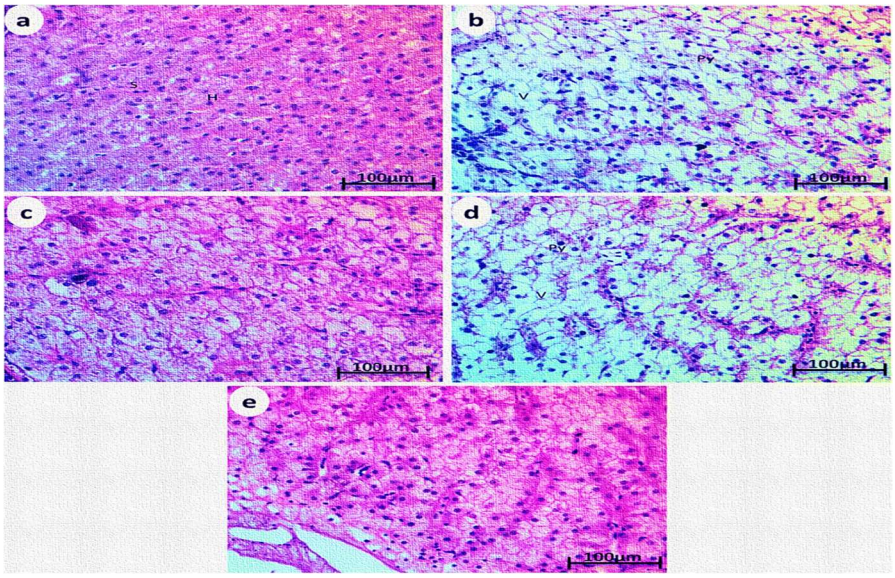

Figure 4: Photomicrographs of Nile tilapia liver histopathology are presented. (a) Displays the normal architecture of liver hepatocytes (H) and blood sinusoids (S). (b) Shows the liver of a fish exposed to copper sulfate, indicating vacuolation (V) and pyknosis (Py). (c) Depicts the liver of a copper sulfate-exposed fish that was pre-fed a basal diet with Spirulina, demonstrating a generally normal histological structure. (d) Illustrates the liver of a fish exposed to copper oxide nanoparticles, also showing vacuolation (V) and pyknosis (Py). (e) Shows the liver of copper oxide nanoparticle-exposed fish pre-fed with Spirulina, revealing a normal histological structure. All sections were stained with hematoxylin and eosin. Scale bar: 50 µm.

4. DISCUSSION

Nanoparticles are increasingly being manufactured and used which will most certainly lead to their increased discharge into aquatic habitats potentially harming fish [30]. In this study we investigated the effects of Copper oxide nanoparticles (CuO-NPs) and $CuSO_4$ on Nile tilapia commonly employed as a bioindicator in environmental research. Fish haematological study recognized as key bioindicators of water quality [31].

The study investigated the impacts of copper sulfate ($CuSO_4$), copper oxide nanoparticles (CuO-NPs) and Spirulina on red blood cell (RBC) counts, hemoglobin levels and various blood parameters. $CuSO_4$ (T_3) and CuO-NPs (T_1) significantly reduced RBC counts and hemoglobin levels due to their toxic effects [32, 33]. In contrast, Spirulina (2.5 gkg^{-1} in T_4 and T_2) maintained RBC and hemoglobin levels similar to the control group, demonstrating its protective role against heavy metal toxicity [34-36]. The treatment with $CuSO_4$ also significantly decreased white blood cell (WBC) counts and platelet levels suggesting cytotoxic effects [37, 38], while Spirulina preserved these parameters[36]. Additionally, $CuSO_4$ reduced hematocrit and Mean Corpuscular Volume (MCV) values, indicating potential disruptions in red blood cell production and size [39]. Spirulina effectively encountered these adverse effects maintaining hematocrit and MCV levels comparable to controls [40]. Overall, the findings underscore for Spirulina's potential to mitigate the toxicity of copper-based treatments. Further research is warranted to explore the underlying mechanisms and optimize these dietary strategies for aquaculture.

Spirulina platensis significantly mitigated the hepatotoxic effects of copper sulfate ($CuSO_4$) in Nile tilapia (*Oreochromis niloticus*), as evidenced by reduced liver enzyme activities [41]. Spirulina ($2.5gkg^{-1}$) maintained AST levels similar to controls, indicating a protective effect against $CuSO_4$ induced liver enzyme changes [29]. For ALT, $CuSO_4$ treatment significantly lowered levels compared to controls potentially due to copper sulfate's modulation of liver enzyme activity [42]. Spirulina ($2.5gkg^{-1}$) maintained ALT levels similar to controls, suggesting it mitigates changes in liver enzyme activity induced by $CuSO_4$. $CuSO_4$ also significantly reduced ALP levels compared to controls, indicating an impact on liver enzyme activity. Spirulina ($2.5gkg^{-1}$) preserved ALP levels suggesting its protective role against $CuSO_4$ induced changes in liver enzyme activity [43, 44]. These results align with findings from Reham Ebaid's study, which reported that pretreatment with A. platensis effectively normalized liver enzyme levels in rats exposed to nano-CuO. Furthermore, research on cadmium-induced hepatic dysfunction in rats demonstrated that green tea extract improved liver function and antioxidant enzyme activities. While Spirulina exhibit protective roles against heavy metal toxicity, Spirulina appears to be effective in reducing liver enzyme levels and enhancing overall liver health, particularly in aquaculture settings. This suggests that Spirulina may offer hepatoprotective benefits, making it a valuable dietary supplement for mitigating the effects of environmental pollutants in fish [45, 46].

Histopathological findings, like hepatocyte vacuolization and other liver damage, were consistent with earlier studies, where similar liver issues were observed in fish exposed to copper compounds. Fish livers are important organs for the metabolism of copper [47] and are frequently used to measure tissue damage from pollutants in the environment [48]. Fish subjected to either copper sulphate ($CuSO_4$) or copper nanoparticles (Cu-NPs) at 100 µg Cu L^{-3} showed dilated hepatic sinusoids and blood buildup in veins in the current investigation [49]. Previous investigations have found similar sinusoid dilatation as a sign of liver injury in fish exposed to copper. Al-Bairuty et al [47], discovered that rainbow trout exposed to $CuSO_4$ at 100 µg Cu L^{-1} experienced cellular necrosis and changed sinusoid spacing, whereas exposure to Cu-NPs generated comparable diseases but damaged a greater area of the liver. It's interesting to note that in this work, $CuSO_4$ produced a larger dilatation of the sinusoids than Cu-NPs, which is in opposition to past research. This disparity could be caused by species variations, length of exposure, or freshwater fish's innate susceptibility to copper toxicity compared to marine fish.

5. Conclusions

In conclusion, both $CuSO_4$ and CuO-NPs induced changes in biochemical and hematological parameters in Nile tilapia. Exposure to these copper compounds also caused changes in the hepatic structure of fish. Overall, CuO-NPs had fewer deleterious effects than $CuSO_4$. Importantly, Spirulina supplementation protected the fish against the potentially deleterious effects of $CuSO_4$ and CuO-NPs via its intrinsic antioxidant activity of their bioactive components. Also, Spirulina may protect against the $CuSO_4$ and CuO-NPs toxicity by its absorption ability for metal ions. Future research should focus on long-term effects and the comprehensive biological pathways involved, which will be essential for developing effective strategies to enhance fish health and sustainability in aquaculture practices.

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