Mitigating Effect of Vitamin-E on Copper Sulphate-Induced Toxicity in African Catfish (*Clarias gariepinus*)

O. I. Azeez and S. F. Braimah

Abstract — Copper sulphate is widely used not only in livestock production especially in the treatment of foot rot in small ruminants but also in aquaculture as algacide and an ectoparasiticide in hatcheries. Meanwhile, it is a common environmental contaminant of water bodies, with carcinogenic, mutagenic and teratogenic effects in humans and animals. The present study was therefore designed to evaluate the toxic effects of copper sulphate and the protective activities of vitamin E on haematological and biochemical parameters as well as oxidative stress status in the African catfish (*Clarias gariepinus*)

Sixty juvenile African catfish with an average weight of 120g were used for the study. They were assigned into six groups (A-F) consisting of ten fish per group. Group A served as the control group and was fed with normal pelletized fish feed only, group B was fed with Vitamin E-supplemented feed only, groups C and D were exposed to copper sulphate (20mg/L and 5mg/L) respectively, plus normal pelletized fish feed while groups E and F were exposed to copper sulphate (20mg/L and 5mg/L), respectively, plus Vitamin E-supplemented feed (240mg/kg feed). Blood samples were collected for haematology and plasma biochemical parameters while gills, liver and kidney samples were collected for evaluation markers of oxidative stress. Exposure to copper sulphate led to a significant decrease in PCV, RBC, Hb concentration, MCV, MCH and total WBC when compared with the unexposed control and those fed with vitamin E-supplemented feed. Furthermore, exposure to copper sulphate caused liver and kidney damages and cell impairment by increasing plasma ALT, AST and ALP activities. It also led to increased oxidative stress as the concentrations of antioxidant endogenous enzymes - GPx, GST and GSH were depleted while potentiating lipid peroxidation and hydroxyl radical generation. The changes in the haematological, biochemical and antioxidant parameters were restored in the fish fed with vitamin E-supplemented feed.

In conclusion, the study showed that exposure to copper sulphate is toxic to African catfish, causing anaemia and liver damage through free radical generation and depletion of antioxidant defence system. Vitamin – E supplementation is therefore recommended during the use of CuSO₄ in aquaculture. Release of the compound to the environment must however be avoided at all cost.

Index Terms — Copper sulphate, Haematology, Oxidative stress, Vitamin E.

I. INTRODUCTION

Contamination of aquatic environments by heavy metals and related compounds through natural and anthropogenic sources has been reported to be a significant threat to public health [1]. It is also known to cause oxidative stress in aquatic organisms leading to numerous physiological dysfunctions in fish [2]. The toxicity of metals becomes

more dangerous when present in high concentrations due to their stability, long half-life and bioaccumulation in the environment [3]. The concentration of copper in pond water remains at a toxic level at least 50 hours after single treatment and can also inhibit the fish growth [4]. Bioaccumulation in the body of organisms, leads to disruption of biological food chain and eventually causing deleterious health effects in the consumers of aquatic products including humans [5], making it important to assess and quantify associated risks of environmental pollution and degradation to aquatic organisms. Heavy metals such as copper affect the vital physiological functions through increased reactive oxygen species (ROS) and free radical generation upon exposure [6].

It was previously reported that heavy metals affect the central nervous function leading to mental disorder, damage the blood constituents and may damage the lungs, liver, kidneys and other vital organs promoting several disease conditions [7]. More so, repeated long-term contact with some of these heavy metals or their compounds may even damage nucleic acids, cause mutation, mimic hormones thereby disrupting the endocrine and reproductive system and eventually lead to cancer [8]. Copper sulphate is released into water as a result of agricultural run-off, natural weathering of soil and discharge from industries [9]. Copper sulphate is extremely toxic to fish and other aquatic organisms, but this toxicity decreases with increasing pH and total alkalinity concentrations [10].

Despite its toxicity, copper sulphate is used for the control of mortality caused by external bacteria, external parasites, and fungus on finfish and finfish eggs in hatcheries [11]. In aquaculture practices, copper sulphate has also been widely used to control algae and other pathogens [12]. The use of copper sulphate in the control of algae and other parasites in water requires high concentrations which are recommended at 50 µg/L minimal dose [13]. However, this practice can pose acute risks to various organisms resulting from direct water application and run-offs from fields adjacent to water bodies [14].

Vitamin E, a fat-soluble vitamin, plays an important role in the maintenance of the normal metabolic processes and physiological functions of aquatic animals [15]. For example, Vitamin E is involved in immune response by potentiating phagocyte system and immunostimulants such as granulocyte macrophage colony-stimulating factor [16]. One of the main physiological functions of Vitamin E is to protect membranes from oxidative damage by mopping up free radicals so as to terminate lipid peroxidation [17, 18]. The antioxidant role of vitamin E is based on the presence of a hydroxyl group in its phenolic group on the chromanol ring that can donate a hydrogen atom and, in this way;

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neutralize a great variety of free radicals including reactive oxygen species [19].

The African catfish (*Clarias gariepinus*) is one of the most important fish in Africa suitable for consumption and commercial purpose. It has recently been adopted as a model animal in the field of biology, vertebrate embryology, and environmental toxicology due to its well documented biology [20], [21] and responses to changes in environmental factors and toxicants.

Although, copper-containing compounds have been subjects of importance in toxicology research, there exists a lack of appropriate scientific data on their effects on *Clarias gariepinus* and possibly the mitigating effects of vitamin E in feed. Therefore, this study examines the toxicity of this compounds and how its toxicity can be ameliorated using Vitamin E.

II. MATERIALS AND METHODS

A. Drugs and chemicals

Analytical grades of copper sulphate (Sigma-Aldrich) and Vitamin E were procured from local agents in Ibadan, Nigeria.

B. Experimental animals

Sixty (60) apparently healthy unsexed juvenile African catfish (*C. gariepinus*), with average weight of 120g procured from the University of Ibadan Aquaculture Laboratory were used for this study. From our observation, the Aquaculture Unit was devoid of any industrial effluent or any other sources of pollution that could affect the biochemical responses of the control fish. The fish were inspected for general fitness and were allowed to acclimatize for 3 weeks in plastic bowls of 80 litres (80 L) capacity during which time they were fed twice daily. All experimental protocols were in compliance with University of Ibadan ethics committee on research in animals as well as internationally accepted guidelines for laboratory animal use and care. The protocol was approved the University of Ibadan Animal Care Use, Research and ethics Committee with ethical clearance number UI-ACUREC/19/0089.

C. Determination of physico-chemical parameters of the water.

The physico-chemical parameters of the water consisting of the temperature, dissolved oxygen (D.O), hydrogen ion concentration (pH) and conductivity were determined before the commencement of the study using Sension M156+ Portable multimeter.

D. Experimental feed

Two types of feed were used for this experiment: a pure commercial feed and a vitamin E-supplemented feed. The commercial pelletized feed (Ecofloat) contains 38% crude protein. The vitamin E-supplemented feed was prepared by mixing the commercial feed with vitamin E (240 mg/kg of feed) and then re-pelletized using a pelletizing machine.

E. Experimental Protocol

After three weeks of acclimatization during which the fish were all fed with the same feed, the fish with similar initial body weight were randomly assigned into 6 groups (A-F) at

the commencement of the treatment. Group A served as the control was fed with normal pelletized fish feed only, group B was fed with Vitamin E-supplemented feed only, group C was exposed to sub-lethal dose of copper sulphate (20 mg/L) [22] with slight modification, plus normal pelletized fish feed, group D was exposed to low dose of copper sulphate (5 mg/L) [22] plus normal pelletized fish feed, group E was administered with copper sulphate (20 mg/L) plus Vitamin E-supplemented feed (240 mg/kg diet) while group F was given to copper sulphate (5 mg/L) plus Vitamin E-supplemented feed (240 mg/kg diet). The fish in each group were fed at 5% body weight twice a day until apparent satiation for fourteen days for fourteen (14) days.

F. Evaluation of growth parameters

All fish were weighed at the commencement and the end of the experiment (14 days), to calculate weight gain (WG) and feed conversion ratio (FCR). The amount of feed consumed was recorded throughout the period of the experiment to determine the feed intake (FI). Growth performance and feed utilization were assessed in terms of weight gain (WG) and feed conversion ratio (FCR) using the following formula:

$$WG (g) = W_2 - W_1$$

$$FCR = \frac{FI}{WG}$$

Where,

W_2 = final body weight,

W_1 = initial body weight,

W = body weight,

FI = feed intake,

WG = weight gain

G. Determination of haematological parameters

After 14 days, blood samples were collected from the ventral vein of each fish into heparinized tubes. From the samples collected, the Packed Cell Volume (PCV) was determined by microhaematocrit method, red Blood Cells (RBC) and White Blood Cells (WBC) haemocytometer method using the improved Neubauer slide [23]. Haemoglobin concentration (Hb) was determined by cyanmethaemoglobin method while the Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated from the PCV, RBC and Hb values [23], erythrocyte osmotic fragility was determined according to the method described by Azeez et al. [24].

H. Determination of plasma biochemical parameters

Blood samples were centrifuged at 4000 rev/min for 10 min to obtain the plasma. From the plasma, urea and creatinine were determined spectrophotometrically according to the methods of Coloumbe and Farreau, and Taussky respectively [25], [26]. Total protein and albumin in plasma samples were also determined by the methods described by Bradford and Doumas et al., [27], [28] while plasma globulin was calculated as the difference between total protein and albumin. Alkaline phosphatase activity was determined by the method of Bessey [29] while activities of ALT and AST were determined by the method of Reitman

and Frankel [30]. The plasma concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were determined by enzymatic colorimetric method using the following Roche kits according to the manufacturer's protocol.

I. Determination of markers of oxidative stress

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi et al. [31], reduced glutathione (GSH) concentrations by the method of Jollow et al. [32] while Glutathione-S-transferase (GST) activity was determined according to the method described by Farombi et al. [33]. Hydrogen peroxide generation was evaluated according to the method of Wolff [34]. Protein concentration was determined by Biuret method as described by Gornal et al. [35]. Glutathione peroxidase activity was measured according to the method described by Rotruck et al. [36].

J. Statistical analysis

All values are expressed as mean $\pm$ S.D. "One-way Analysis of Variance" (ANOVA) with Tukey's post-hoc test was performed to compare the data between groups using GraphPad Prism version 7.0 with probability value of $P < 0.05$ considered statistically significant.

III. RESULTS

A. The physico-chemical parameters of the water used

From the results in Table 1, the physico-chemical parameters of the water used were within the accepted international EPA standard. For example, the pH of the water was neutral at 7.33 ± 0.84 , which corresponds to the average acceptable international EPA standard. The dissolved oxygen was also high enough for survival of the fish at 5.71 ± 0.88 , which was higher than the acceptable international EPA standard.

TABLE 1: THE PHYSICO-CHEMICAL PARAMETERS OF THE WATER USED

Parameters	Values	EPA standard
Temperature ($^{\circ}\text{C}$)	27.34 ± 2.67	25
pH	7.33 ± 0.84	$\geq 6 \leq 9$
Dissolved oxygen (mg/L)	5.71 ± 0.88	≥ 5
Conductivity ($\mu\text{S/cm}$)	215.53 ± 13.01	200-1000

EPA – Environmental Protection Agency.

B. Growth performance and feed conversion

Figure 1 shows the growth performance and feed utilization parameters of the African catfish exposed to copper sulphate. After 14 days of exposure, there was a reduction in weight in the fish exposed to sub-lethal dose

(20 mg/L) of CuSO_4 whereas those fish in all the other groups showed increases in weight. The feed conversion ratio and efficiency was highest in group B that was fed vitamin E-supplemented feed alone, closely followed by group F that was exposed to copper sulphate (5 mg/L) plus vitamin E-supplemented feed while group C did not show any evidence of feed conversion but lost weight instead with an FCR of -2.4.

Table 2 shows the weight of liver and heart compared to the body weight of African catfish in acute copper sulphate exposure. The percentage weight of liver to body ratio of fish exposed to different concentrations of copper sulphate (20 mg/L and 5 mg/L) relative to body ratio was significantly higher than those of the control group and the groups fed with vitamin E-supplemented feed at $p < 0.05$.

Fig. 1: Effect of acute copper sulphate toxicity on percentage weight gain (A) and feed conversion ratio (B) in African catfish (*Clarias gariepinus*) as modulated by vitamin E. A = Control, B = Vitamin E-supplemented feed, C = Copper sulphate (20 mg/L) alone, D = Copper sulphate (5 mg/L) alone, E = Copper sulphate (20 mg/L) alone + Vitamin E-supplemented feed, F = Copper sulphate (5 mg/L) alone + Vitamin E-supplemented feed, n = number of fish.

TABLE 2: EFFECT OF ACUTE COPPER SULPHATE TOXICITY ON ORGAN-BODY WEIGHT RATIO IN AFRICAN CATFISH (*CLARIA GARIEPINUS*) AS MODULATED BY VITAMIN E

Parameters	A	B	C	D	E	F
BW (g)	186.23 ± 12.55^a	192.13 ± 14.86^{bc}	141.19 ± 13.64^{abcde}	163.20 ± 11.76	165.46 ± 12.38^c	174.90 ± 9.37^d
LW (g)	2.31 ± 0.18	2.11 ± 0.23	2.46 ± 0.15	2.66 ± 0.20	2.19 ± 0.17	2.29 ± 0.62
L-B weight (%)	1.24 ± 0.04^{ab}	1.10 ± 0.05^{cd}	1.74 ± 0.23^{acef}	1.63 ± 0.12^{bd}	1.33 ± 0.09^e	1.31 ± 0.07^f
HW (g)	0.16 ± 0.05	0.15 ± 0.04	0.14 ± 0.02	0.14 ± 0.02	0.17 ± 0.04	0.14 ± 0.01
H-B weight (%)	0.08 ± 0.02	0.09 ± 0.01	0.10 ± 0.02	0.08 ± 0.01	0.10 ± 0.02	0.10 ± 0.00

Data are presented as mean $\pm$ SD. Where BW = bodyweight (g), LW = liver weight (g), L-B = percentage liver/body ratio (%), HW = heart weight (g), H-B = percentage heart/body ratio. Values with the same superscript alphabets along the same row are significantly different at $P < 0.05$. A = Control, B = Vitamin E-supplemented feed, C = Copper sulphate (20 mg/L) alone, D = Copper sulphate (5 mg/L) alone, E = Copper sulphate (20 mg/L) alone + Vitamin E-supplemented feed, F = Copper sulphate (5 mg/L) alone + Vitamin E-supplemented feed, n = number of fish.

C. Haematological parameters

As shown in Table 3, exposure of *C. gariepinus* to copper sulphate caused significant decreases ($P<0.05$) in PCV, RBC, Hb, MCV and MCHC values when compared with the control group, whereas in the groups co-treated with copper sulphate and vitamin E, haematological parameters were similar to those in the control and significantly higher than the groups exposed to copper sulphate alone. However, MCH values showed no significant difference across the groups. The total WBCs, lymphocyte and neutrophil count decreased significantly ($p<0.05$) in blood of the exposed fish when compared with the control as seen in Table 4, while that of the fish fed with vitamin E supplemented feed increased significantly when compared with those exposed

to copper sulphate alone. There was no significant difference in the monocyte count across the groups.

Acute exposure to copper sulphate also affected the erythrocyte osmotic resistance in hypotonic solution as seen in Fig. 2. For example, at 0% NaCl concentration, the erythrocyte osmotic fragility of the fish in group C that was exposed to copper sulphate (20mg/L) alone was significantly higher ($p<0.05$) than that of group F that was exposed to copper sulphate (5mg/L) plus vitamin E-supplemented feed. At 0.5% NaCl concentration, the fish in group C had significantly lower ($p<0.05$) erythrocyte osmotic fragility compared to the fish in groups A, D, E and F. Furthermore, at 0.7% NaCl concentration, the erythrocyte osmotic fragility of groups D and F was significantly higher ($p<0.05$) than the fish in group C.

TABLE 3: EFFECT OF COPPER SULPHATE ON ERYTHROCYTE PARAMETERS OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) AS MODULATED BY VITAMIN E

Parameters	A	B	C	D	E	F
PCV (%)	47.20±8.08 ^a	43.40±3.85 ^b	30.20±5.81 ^{abcd}	39.00±2.24	46.00±3.32 ^c	40.00±4.53 ^d
Hb (g/dl)	10.80±1.66 ^a	10.38±1.50 ^b	7.66±1.44 ^{abc}	9.34±1.61	10.44±0.85 ^c	9.25±0.35
Rbc (x10 ⁶ /μL)	3.55±0.38 ^{ad}	3.61±0.35 ^{be}	2.28±0.22 ^{abc}	2.21±0.20 ^{def}	3.44±0.78 ^{cf}	3.01±0.40
MCV	236.74±37.19 ^{ae}	268.40±19.51 ^{bf}	133.20±27.56 ^{abcd}	177.60±20.39 ^{ef}	212.84±30.40 ^e	227.73±32.11 ^d
MCH	45.60±10.21	41.46±9.72	33.87±6.94	42.88±10.73	29.46±4.08	46.74±17.95
MCHC	25.64±2.11 ^a	25.04±1.77 ^{bd}	20.65±1.92 ^{abcd}	21.84±1.41	24.12±2.09	24.33±1.78 ^c

Values are presented as mean ± SD while values with the same superscript alphabets along the same row are significantly different at $P<0.05$. A = Control, B = Vitamin E-supplemented feed, C = Copper sulphate (20 mg/L) alone, D = Copper sulphate (5 mg/L) alone, E = Copper sulphate (20 mg/L) alone + Vitamin E-supplemented feed, F = Copper sulphate (5 mg/L) alone + Vitamin E-supplemented feed, n = number of fish.

TABLE 4: EFFECT OF COPPER SULPHATE ON ERYTHROCYTE PARAMETERS OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) AS MODULATED BY VITAMIN E

Parameters	A	B	C	D	E	F
TWBC (x10 ⁶ /μL)	1.20±0.07 ^{ab}	1.23±0.01 ^{cd}	0.40±0.09 ^{acefg}	0.90±0.09 ^{bdehi}	1.22±0.15 ^{efh}	1.30±0.14 ^{dgi}
Lymph. (μL)	657.00±84.16 ^a	782.80±40.31 ^{be}	309.00±66.34 ^{abcd}	484.80±120.60 ^{ef}	787.50±157.60 ^{cf}	596.00±168.60 ^d
(%)	(79.40±5.18)	(78.60±6.54)	(80.00±5.10)	(74.00±9.87)	(70.80±2.17)	(48.40±5.23)
Neut. (μL)	109.20±12.10 ^a	125.80±13.25 ^{bcd}	62.30±9.50 ^{abef}	83.40±15.86 ^c	96.60±17.23 ^{de}	104.80±18.10 ^f
(%)	(10.80±5.98)	(15.60±6.73)	(2.20±1.79)	(16.80±9.52)	(17.20±2.78)	(41.80±7.53)
Mono. (μL)	91.50±12.48	63.30±25.72	84.70±11.89	71.50±28.43	120.60±50.39	97.30±24.27
(%)	(9.80±2.86)	(5.80±2.78)	(17.80±5.02)	(9.00±3.54)	(12.00±2.55)	(9.80±6.94)

Values are presented as mean ± SD while values with the same superscript alphabets along the same row are significantly different at $P<0.05$. A = Control, B = Vitamin E-supplemented feed, C = Copper sulphate (20 mg/L) alone, D = Copper sulphate (5 mg/L) alone, E = Copper sulphate (20 mg/L) alone + Vitamin E-supplemented feed, F = Copper sulphate (5 mg/L) alone + Vitamin E-supplemented feed, n = number of fish, TWBC=Total white blood cells, Lymph = Lymphocytes, Neut = Neutrophils, Mono = Monocytes.

Fig. 2. Erythrocytes osmotic fragility of African catfish (*Claria gariepinus*) exposed to acute copper sulphate toxicity and concurrent treatment with vitamin E. Values are expressed as mean ± SD and n is 5 for each group.

D. Plasma biochemical parameters

The effects of acute copper sulphate toxicity on plasma biochemical parameters in African catfish are presented in Table 5. The activity of aspartate transaminase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) increased significantly ($p < 0.05$) in group C that was exposed to copper sulphate (20mg/L) but this increase was mitigated/corrected in groups co-treated with vitamin-E. The urea and creatinine levels in the groups exposed to 20mg/L and 5mg/L of copper sulphate was significantly lower

($P < 0.05$) when compared with the control. The altered urea and creatinine levels were corrected in the groups co-treated with Vitamin-E. The plasma protein profile was altered as a result of copper sulphate exposure. The results showed a significant reduction ($p < 0.05$) in total protein and globulin levels in the fish exposed to copper sulphate especially those exposed to 20mg/L copper sulphate while albumin was insignificantly reduced. Co-treatment with copper sulphate and vitamin E-supplemented feed improved the plasma protein levels even better than the control values.

TABLE 5: EFFECTS OF ACUTE COPPER SULPHATE TOXICITY ON PLASMA BIOCHEMICAL PARAMETERS IN THE AFRICAN CATFISH (CLARIAS GARIEPINUS) AS MODULATED BY VITAMIN E

Parameters	A	B	C	D	E	F
AST (IU/L)	12.64±2.00 ^a	12.33±1.79 ^b	18.67±2.52 ^{abcd}	13.33±1.53	13.00±1.27 ^c	12.94±2.52 ^d
ALT (IU/L)	9.33±1.53 ^a	8.47±1.24 ^b	14.67±1.53 ^{abcd}	11.67±2.08	8.33±1.53 ^c	9.33±2.08 ^d
ALP(IU/L)	42.67±3.64 ^a	40.67±3.07 ^b	55.11±4.00 ^{abc}	48.33±6.66	44.33±3.43	43.22±2.49 ^c
Urea (mg/dl)	26.67±3.06 ^a	27.67±3.51 ^b	18.67±1.53 ^{abcd}	23.33±1.53 ^e	30.33±2.52 ^{ce}	25.67±2.08 ^d
Creatinine (mg/dl)	0.87±0.12 ^{abc}	0.80±0.10 ^{de}	0.43±0.06 ^{adf}	0.53±0.06 ^{be}	0.73±0.06 ^f	0.60±0.10 ^c
Plasma proteins (g/dl)	6.77±0.18 ^a	6.87±0.25 ^b	6.03±0.21 ^{abcd}	6.57±0.31	7.13±0.12 ^c	6.87±0.25 ^d
Albumin (g/dl)	3.73±0.32	3.90±0.27	3.50±0.10	3.73±0.31	4.17±0.12	3.80±0.30
Globulin (g/dl)	3.93±0.24 ^{abc}	3.97±0.12 ^{def}	2.53±0.29 ^{adgh}	3.01±0.12 ^{bei}	3.34±0.10 ^{cfe}	3.57±0.14 ^{hi}
Total cholesterol (mg/dl)	164.70±5.51	155.00±7.94	163.30±7.02	158.70±11.93	151.30±12.22	171.00±12.53
Triglycerides(mg/dl)	70.00±12.53	57.33±4.73	72.67±12.58	66.00±22.61	57.33±15.50	78.67±27.21
HDL (mg/dl)	44.67±2.89	40.33±3.51	45.00±4.00	45.00±4.36	41.33±5.51	48.00±5.57
LDL (mg/dl)	135.00±9.64	120.70±10.50	142.30±3.51	136.30±12.01	126.00±12.29	147.70±15.57

Values are presented as mean ± SD while values with the same superscript alphabets along the same row are significantly different at $P < 0.05$. A = Control, B = Vitamin E-supplemented feed, C = Copper sulphate (20mg/L) alone, D = Copper sulphate (5mg/L) alone, E = Copper sulphate (20mg/L) alone + Vitamin E-supplemented feed, F = Copper sulphate (5mg/L) alone + Vitamin E-supplemented feed, n = number of fish.

E. Markers of oxidative stress

The effects of acute copper sulphate toxicity on markers of oxidative stress in the gills (Fig. 2), liver (Fig. 3) and kidneys (Fig. 4) of African catfish and the modulatory effect of vitamin E are shown below. As shown in figure 3, the glutathione peroxidase (GPx), glutathione-S-transferase (GST) and reduced glutathione (GSH) of groups exposed to copper sulphate (20 mg/L and 5 mg/L) were significantly lower ($p < 0.05$) than that of the control group. The hydrogen peroxide generation (H_2O_2) and malondialdehyde (MDA) of the fish exposed to copper sulphate were significantly higher ($p < 0.05$) than that of the control group. Supplementation with vitamin E restored the altered parameters close to the values obtained in the control group. Fig. 4 and 5 show the effect of acute copper sulphate toxicity on markers of oxidative stress in the liver and kidney of African catfish respectively and the modulatory effect of vitamin E. In the groups exposed to copper sulphate (20 mg/L and 5 mg/L), the activities of the enzymes GPx, GST and GSH were significantly lower ($p < 0.05$), while the malondialdehyde and hydrogen peroxide generation were significantly higher ($p < 0.05$) than that of the control groups. In the liver, the total protein concentration was significantly lower ($p < 0.05$) in the group exposed to 20mg/L of copper sulphate than the control group and the group fed with vitamin E-supplemented feed, but there was no significant difference in the total protein level in the kidney. Supplementation with vitamin E increased the total protein level, GPx, GST and GSH activities while decreasing the hydrogen peroxide generation and lipid peroxidation better than the toxicant groups.

IV. DISCUSSION

The study demonstrated the toxic effect of copper sulphate, which was evidenced by the significant reduction in the growth performance parameters (decrease in body weight, weight gain and feed conversion ratio (which is an indicator of feed efficiency) as seen in the fish exposed to different doses of copper sulphate only. The observed reduction in body weight was probably due to high energy demand and reduced feed consumption in fish exposed to toxic chemicals. For example, it was reported that exposure to toxicants results in reduced body weight due to reduced feed consumption [37]. In the present study, the growth parameters improved significantly in vitamin E-supplemented groups compared to control group. This is also in agreement with the report of Hossein et al., [38] who recorded an increase in growth parameters in vitamin E-supplemented capisan brown trout fish following exposure to different doses of vitamin E and C.

Copper sulphate increased the relative liver weight when compared with the control. The elevation in the relative liver weight is thought to be due to reduced body weight caused by copper sulphate while the weight of the liver remained unchanged or hepatocellular hyperplasia and hypertrophy in response their exposure to the toxicant [39]. But in fish fed with vitamin E, the relative liver weight was not affected. This was probably due to the activity of vitamin E, which is a biological antioxidant that could contribute to improved growth because of its ability to neutralize free radicals and reduce lipid peroxidation in the plasma, liver and muscles [40], [41]. It is generally accepted that apart from microbial spoilage, lipid oxidation is the primary process by which

quality loss of muscle occurs in food animals, thereby resulting in weight loss [42].

Fig. 3: Effect of acute copper sulphate toxicity on markers of oxidative stress in the gills of African catfish as modulated by vitamin E. A = Control, B = Vitamin E-supplemented feed, C = Copper sulphate (20 mg/L) alone, D = Copper sulphate (5 mg/L) alone, E = Copper sulphate (20 mg/L) alone + Vitamin E-supplemented feed, F = Copper sulphate (5 mg/L) alone + Vitamin E-supplemented feed. Values with the same superscript alphabets are significantly different at $p < 0.05$.

Fig. 4: Effect of acute copper sulphate toxicity on markers of oxidative stress in the liver of African catfish as modulated by vitamin E. A = Control, B = Vitamin E-supplemented feed, C = Copper sulphate (20 mg/L) alone, D = Copper sulphate (5 mg/L) alone, E = Copper sulphate (20 mg/L) alone + Vitamin E-supplemented feed, F = Copper sulphate (5 mg/L) alone + Vitamin E-supplemented feed. Values with the same superscript alphabets are significantly different at $p < 0.05$.

Fig. 5: Effect of acute copper sulphate toxicity on markers of oxidative stress in the kidney of African catfish as modulated by vitamin E. A = Control, B = Vitamin E-supplemented feed, C = Copper sulphate (20mg/L) alone, D = Copper sulphate (5mg/L) alone, E = Copper sulphate (20mg/L) alone + Vitamin E-supplemented feed, F = Copper sulphate (5mg/L) alone + Vitamin E-supplemented feed. Values with the same superscript alphabets are significantly different at $p < 0.05$.

Haematological profile has been known to be a good indicator of the physiological variations after exposure to pollutants and other pathophysiological conditions, reflecting the overall health status of fish [43]. In the current study, acute exposure of African catfish to copper sulphate resulted in microcytic hypochromic anaemia as evidenced by a significant decrease in the PCV, RBC, haemoglobin, MCV and MCHC values which were corrected by vitamin E. The decrease in PCV, RBC and haemoglobin values indicate deterioration of the condition of the fish and development of anaemia. These reductions after exposure to pollutants may be attributed to the inhibition of erythropoiesis or an increased rate of erythrocyte destruction in the blood or in haematopoietic organs [44]. Haemoglobin concentration reveals the status of an organism's oxygen carrying capacity and the organism itself tries to maintain them as much as possible in the face of any stressor. Reduction of haemoglobin affects the oxygen carrying capacity [45] and poses a serious challenge to the survival of fish in aquatic environment [46]-[48]. The reduced haematological parameters were corrected in the fish fed with vitamin E-supplemented feed. The enhancing action of vitamin E on fish haematology has been attributed to its role as antioxidant protecting cell membranes including red blood cells against oxidative damages [49].

Erythrocyte osmotic fragility reveals the tendency of erythrocytes to haemolyse or rupture under stress and is affected by several factors including membrane composition and integrity as well as cells' sizes and surface area to volume ratio [50]. Due to the damaging effects of free radicals and reactive oxygen species on erythrocyte membrane proteins and lipids resulting in increased fluidity and susceptibility to osmotic lysis, erythrocyte osmotic fragility has been used as a marker of oxidative stress induced by several factors such as aging [24], exercise [51],

transportation stress [52] and haemodialysis [53]. In the present study, exposure to copper sulphate led to an increase in erythrocytes osmotic fragility except for the fish exposed to sub-lethal dose of copper sulphate (20mg/L) that had very low erythrocytes osmotic fragility. These low erythrocytes osmotic fragility could be attributed to bone marrow response to anaemia leading to the release of large amount of reticulocytes, which are more osmotically stable into circulation. According to Robert [54], reticulocytes are immature anucleated red cells containing blue-stained granules which are more resistant to osmotic lysis due to the presence of mitochondria which pumps water out of the cell.

Exposure to copper sulphate resulted in leukopenia due to reduction in leucocyte values in the exposed fish. The decreased number of white blood cells (leukopenia) may be due to generalized injury caused to the haematopoietic stem cells in the bone marrow and other erythropoietic organs by exposure to heavy metals [55]. Similar disruption in haematopoietic mechanisms was observed by Adjroud [56] after potassium dichromate exposure in both male and female rats. This was corrected in the fish fed with vitamin E-containing feed where the leucocyte values were elevated. This activity of vitamin E may not be unconnected with its antioxidant effect and its role as a free radical scavenger. Vitamin E play a vital role in scavenging lipid peroxy radical [57] and maintenance of fish immunity [58]. Vitamin E has also been considered as an activator of phagocyte population and immunostimulants [16].

Plasma biochemical parameters such as urea and creatinine are biomarkers for liver and kidney function and serve as useful indicators of stress impacts caused by pollutants [59]. In the present study, urea and creatinine levels were observed to be significantly lower in the fish exposed to copper sulphate when compared to the control. This could be as a result of liver damage because these

metabolites are produced in the liver and low levels indicate that the liver was probably unable to make the normal amount of these metabolites [60].

Plasma enzymes, AST, ALT and ALP are found in several fish tissues, such as heart, kidneys, liver, brain, erythrocyte, intestine and gills. More specifically, AST and ALT enzyme activities are predominant in the liver and the cardiac tissues; therefore alterations in their activities could indicate liver injury and cardiotoxicity [61]. Increased AST, ALT and ALP activity has also been observed in liver and kidney impairment and during exposure to toxic metals and xenobiotics [62]. Therefore, the release of these intracellular enzymes into the bloodstream and their increased activity in plasma are important biomarkers for cell impairment or degenerative changes [63]. Enzymes such as ALT and AST, localized within the cells of numerous organs, including the liver, act as significant indicators for evaluation of organ status in cases of injury, tissue damage or organ dysfunction [64]. Accordingly, the results presented in the present study indicate significant increases in AST, ALT and ALP activities in fish exposed to copper sulphate when compared with the control while concomitant exposure to vitamin E led to significant decreases in ALT, AST and ALP levels, indicating protection from liver damage by vitamin E. Increase in these liver enzymes was similar to the observations of Zaghloul et al. [65] who exposed three species of fish, namely *Oreochromis niloticus*, *Tilapia zillii*, and *Clarias gariepinus* to copper metal for 30 days and recorded a significant increase in the levels of ALP, AST, and ALT enzymes. He attributed the elevated liver enzymes to liver damage by the heavy metal as a result of major pathologic changes in permeability of cell membrane or hepatic cell rupture.

The present investigation showed a significant decrease in the plasma total protein level (hypoproteinemia) after exposure to copper sulphate. Such decrease of total protein may be due to destruction of protein synthesizing subcellular structures, inhibition of hepatic synthesis of plasma protein as a result of liver cell damage, heavy metals-protein interaction or protein catabolism to provide extra energy requirement to overcome the stress in the polluted medium [66].

It is interesting to note that oxidative stress is a major hallmark of the findings on enzymatic and non-enzymatic antioxidant activities in the gills, liver and kidney of African catfish in the present study. Our findings showed that the reduced glutathione (GSH), Glutathione peroxidase (GPx) and Glutathione-S-Transferase activities in the gills, liver and kidney of African catfish exposed to copper sulphate were significantly lower when compared to those fed with vitamin E-supplemented feed. The reduction could be due to the exhaustion of these antioxidants as a result of the increased production of free radicals. The inhibition of the GPx activity by pesticides and copper sulphate has been reported in various studies in fish species including that of Yonar et al. [67] who reported that GPx activity in liver and gill of rainbow trout was decreased in copper sulphate-induced groups. Sayeed et al. [68] also reported that the exposure to deltamethrin caused a significant decrease in the activity of GPx in *Channa punctatus* gills. The reduced GST activity was in conformation with the work of Arojojoye et

al., [69] who observed a lowered GST activity in African catfish exposed to heavy metals while reduction in the GSH content was also in agreement with the work of Heba [70] after the exposure of female catfish to endosulfan. Supplementation with vitamin E showed a significant improvement in the concentrations of these antioxidants in the gills, liver and kidneys of the exposed fish.

Total protein also used as oxidative stress biomarker was reduced in the gills and liver with the reduction more pronounced in the liver of those exposed to copper sulphate (20mg/L). This reduction indicates the destructive effect of copper sulphate due to an increase in the generation of reactive oxygen species. This effect was mitigated in the group fed with vitamin E-supplemented feed.

Copper sulphate exposure also led to a significant increase in lipid peroxidation (MDA) and hydrogen peroxide generation in the gills, livers and kidneys of exposed fish. The increase in MDA levels in this study is in line with the work of Yonar et al. [67] who observed an increase in MDA levels in rainbow trout exposed to copper sulphate. The level of lipid peroxidation and hydrogen peroxide generation was significantly decreased in copper sulphate-exposed fish fed diets supplemented with vitamin E.

V. CONCLUSION

Copper sulphate, although used, as algacide in aquaculture and agriculture is toxic to fish via increased oxidative stress as a result of generation of free radicals and other ROS and depletion of antioxidant defence. The results obtained in this study revealed that oxidative stress may, in part, be contributing to the copper sulphate-induced hepatic, renal and gill damage while dietary supplementation with vitamin E conferred protective effects against its toxicity. After exposure to this algacide, the ability of vitamin E to improve and recover the haematological and biochemical parameters as well as protection against reactive oxygen species were clearly seen in the fish fed with vitamin E-supplemented diet. As a result, vitamin E modulates and diminishes the adverse effects of copper sulphate toxicity thereby improving their physiological status and in turn raising their resistance to oxidative stress. Thus, the use of vitamin E in diet could safely be recommended whenever the use of copper sulphate for therapeutics is inevitable in fish.

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