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Possible ameliorative effect of dietary curcumin against water copper toxicity in *Clarias gariepinus* fish

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ABSTRACT

A total of seventy-two healthy Nile catfish; *Clarias gariepinus* were distributed into four distinct groups. These groups were fed either a commercial basal diet or a diet enriched with 500 mg curcumin/kg ration for 12 weeks. Among these groups, two groups were exposed to a sublethal concentration of copper (0.35 mg/l) and were fed a normal diet or a curcumin-enriched diet as an antioxidant. The other two control groups were comprised of fish raised in dechlorinated tap water and were fed either the commercial basal diet or the curcumin-supplemented diet for the same 12-week experimental period. The study's findings revealed that *Clarias gariepinus*, when subjected to a sublethal copper concentration (0.35 mg/l), accumulated a greater amount of copper in their vital organs (gills, liver, and muscles) after 12 weeks. This accumulation coincided with a decrease in fish's rate of growth and quality of meat (indicated by higher muscle water content and ash but lower muscle total protein and lipids), a reduction in RBCs count, Hb content, and Ht values, and an increase in WBCs count, serum glucose, creatinine, uric acid, total protein, albumin, globulin levels, A/G ratio, and AST, ALT and ALP activities when compared to the control group of fish with a highly significant difference ($P < 0.01$). However, fish that were exposed to copper toxicity and fed curcumin supplemented diet (500 mg/kg diet) exhibited values that were more or less similar to those of the control group of fish that were reared in dechlorinated tap water and fed a commercial basal diet or curcumin supplemented basal diet (500mg/kg diet).

INTRODUCTION

The aquatic environment subjected to different types of pollutants of industrial, domestic and agricultural wastes and severely affect aquatic organisms **(Ali *et al*., 2019; Abdel-Khalek *et al*., 2020)**. Heavy metals and other harmful and dangerous compounds released by industries have a significant impact on aquatic ecosystem. Due to their toxicity and accumulative nature, heavy metals discharge into the aquatic environment has the potential to alter the biodiversity of the aquatic species as well as aquatic ecosystems **(Abdel-Khalek *et al*., 2016; Briffa *et al*., 2020 ; Garai *et al*., 2021)**.

Even though copper is a trace element that should only be present in very small amounts, it is discharged into fresh water environments in large amounts as an industrial effluent, which has a negative impact on the freshwater fauna, particularly fish **(Zaghloul *et al*., 2006; Johnson *et al*., 2007; Mcintyre *et al*., 2008; Monteira *et al*., 2009; Kong *et al*., 2013; Abdel-Khalek *et al*., 2020).**

Industrial fish farming is often associated with the production of unhealthy cultured fish, according to research **(Bondad-Reantaso *et al*., 2005)**. Addressing the issue of suboptimal health in such fish has become a crucial area of concern, and nutritional strategies are now being implemented. One such strategy is the use of various feed additives, such as organic and inorganic acids, feed enzymes, pre- and probiotics, and essential oils, which have been demonstrated to enhance immunological response, physical barrier function, and digestive function in the aquaculture industry **(Lall and Kaushik, 2021)**.

Applying an external source of antioxidants may provide some oxidative stress protection. The term "antioxidant" refers to a broad range of substances that can donate electrons and combat free radicals, preventing cellular damage. Research has started to show the benefits of using phytochemicals and herbal items as safe, natural substances with antioxidant action in fish production **(Chakraborty and Hancz, 2011; Rawling *et al*., 2009)**.

One of the best-studied natural compounds is curcumin, a polyphenolic phytochemical extracted from the root of the turmeric plant (*Curcuma longa*). It has a wide range of potential therapeutic or preventive effects, including antioxidant, anticancer, antibacterial, hepatoprotective and gastroprotective effects **(Soudamini and Kuttan, 1989; Ruby *et al*., 1995; Singh and Khar, 2006). Sruthi *et al*. (2018)** reported that curcumin, is a safe and effective feed supplement that boosts nutrient uptake and growth of *Oreochromis mossambicus* and aids in feed management by minimizing feed consumption while maximizing growth output.

Since every form of life depends on water, water pollution is a big problem. Many of the areas in which fish inhabit today are threatened by pollutants, including copper, the most abundant heavy metal in any aquatic habitat. It is imperative to find a protective agent against metal toxicity. Therefore, the aim of this study was to investigate the effects of long-term (12 weeks) exposure to sublethal concentrations of copper (0.35 mg/l, 1/10 LC50) on the biological effects and physiological status of *Clarias gariepinus* fish fed a commercially available basal feed or a basal feed supplemented with curcumin (500 mg/kg ration).

MATERIALS AND METHODS

Seventy-two healthy Nile catfish, *Clarias gariepinus*, weighing about 50 grams each, were caught from a fish farm in Fayoum Governorate, Egypt. The fish were transferred to the wet laboratory of the Department of Zoology, Fayoum University, Faculty of Science and acclimatized for two weeks in aquariums with well-ventilated dechlorinated tap water at temperature (25±10°C). The oxygen concentration was maintained at 7.2 mg/l using air pump.

Determination of copper half lethal concentration LC_{50}:

According to **Litchfield and Wilcoxon (1949)**, copper toxicity studies on *Calarias gariepinus* (50 g/fish roughly) were performed. The experiments revealed that the Nile catfish, *Clarias gariepinus*, has a 96-hour LC_{50} value for copper of 3.5 mg/l.

Diet formulation:

The experimental fish ration was formulated according to **Abdel-Tawwab and Abbass (2017)**. The main constituent of fish ration is crude protein, all diets contained 32% crude protein. According to previous study by **Abdel-Tawwab and Abbass (2017)**, the obtained commercial Curcumin obtained from a local mark contained moisture 5.7%, crude protein 8.7%, total lipids 3.9%, total carbohydrates 68.3%, and total ash 7.6%. Curcumin was added to the ingredients of each diet to represent 0.0 (control) or 5.0 g/kg diet.

The experimental Design:

Seventy-two *Clarias gariepinus* fish were distributed in 120-liter glass aquariums, 6 fish per aquarium, and divided into 4 groups of 18 fish each. To prevent the accumulation of metabolites, dechlorinated tap water was used throughout the experiments and the water was changed twice a week. Fish received the following treatments for 12 weeks to monitor the effect of sublethal concentrations (1/10 LC_{50}) of copper in two supplemented staple food levels (0 and 500 mg curcumin/kg fish diet):

Group I: Control group, fish reared in dechlorinated tap water and fed on 0.0 mg curcumin fish ration.

Group II : Fish reared in dechlorinated tap water and fed on curcumin supplemented fish ration (500 mg /kg fish ration).

Group III : Fish were exposed to 0.35 mg Cu /l) and fed on 0.0 mg curcumin fish ration.

Group IV: Fish were exposed to 0.35 mg Cu /l and fed curcumin supplemented fish ration (500 mg/kg fish ration).

The fish were fed twice a day at a rate of 3% of the live weight of the fish. The fish were weighed every two weeks to monitor their growth rate and adjust their food intake. Blood samples were collected at the end of the experimental period (12 weeks) before the fish were sacrificed to obtain organ samples and their muscles were chemically analysed to determine their protein, lipid, ash and water content.

(1) Residual copper in fish tissues:

Copper bioaccumulation in gills, liver and muscles of *clarias gariepins* exposed to the different studied treatments of the experiment was detected according to **APHA (2005)** and measured using atomic absorption spectrophotometry (Perkin Elmer, 2280).

(2) Growth indices:

To optimize the artificial feed rate, fish body weights were measured to the nearest gram and total body length to the nearest 0.1 cm for the various study groups. The following growth indices were established at the conclusion of the trial period (12 weeks).

Specific growth rate:

Specific growth rate (growth rate/day) was determined according to the equation postulated by Allen and Wooton (1982):

$$S.G.R. = \frac{Ln\ Wf - Ln\ Wo}{(Tf - To)} \times 100$$

Where: *Wf* : The final weight of fish in g , *Wo* : The initial weight of fish in g,
(Tf –To): Time between the final and initial weight in days, *Ln* : Logarithm to base.

Condition factor (k):

K factor was calculated for individual fish from the formula recommended by Schreck and Moyle (1990):

$$K = \frac{W}{L^3} \times 100$$

Where:

W : is the wet weight in g.

L : is the total length in cm.

(3) Blood sampling and examination

Sodium citrate was used as an anticoagulant to withdraw blood samples from the arteria caudalis. It was discovered that heparin and oxalate did not entirely prevent coagulation **(Hesser, 1960 and Falkner and Houston, 1966)**.

Blood samples were examined immediately for the following blood constants:

i) Count of Red and White blood cells:

Total number of erythrocytes (RBCs) and leukocytes (WBCs) were counted using improved Neubauer Haemocytometer.

ii) Haemoglobin content:

Haemoglobin content was estimated using cyanmethemoglobin method described by **Van Kampen and Zijlstra (1961)**.

iii) Haematocrit value (PCV):

Packed cell volume (PCV) was carried out in small haematocrit tubes using haematocrit centrifuge at 3000 r.p.m. for 15 minutes.

iv) Blood derivatives were calculated according to Gupta (1977) as follows:

(a) Mean corpuscular volume (μm^3/cell)

$$MCV = \frac{PCV\ (\%)}{RBCs\ (million/mm^3)} \times 10$$

(b) Mean corpuscular haemoglobin (pg/cell)

$$MCH = \frac{Hb\ (gm/100\ ml\ blood)}{RBCs\ (million/mm^3)} \times 10$$

(c) Mean corpuscular haemoglobin concentration (g/100 ml)

$$MCHC = \frac{Hb\ (gm/100\ ml\ blood)}{PCV} \times 100$$

(4) Biochemical analysis

(A) Serum analysis:

Serum of blood samples collected from fish of the studied treatments, were obtained by centrifuge blood at 3000 r.p.m. for the following analysis:

- **Blood glucose estimation:** Blood glucose levels (mg/dl) were measured using SPECTRUM kits by GOD-PAP enzymatic colorimetric method at (492- 550nm), Colour change is then measured spectrophotometrically according to Weibel and Bright (1971).

- **Serum AST (E.C.2.6.1.1.) , ALT (E.C.2.6.1.2.) and ALP:** Serum aspartate amino transferase (AST, E.C.2.6.1.1.) and alanine amino transferase (ALT, E.C.2.6.1.2.) and alkaline phosphatase (ALP) activities were investigated colourimetrically using transaminases kits according to Reitmans and Frankel (1957).

- **Serum creatinine:** Creatinine was measured using colorimetric method of Henry (1974).

- **Serum uric acid:** According to **Barham and Ttinder (1972)**, serum uric acid Uric acid was measured using enzymatic reaction.

- **Protein profile estimation:** Total protein was tested using Bradford (1976) techniques that Spector adapted. Using the spectrum from a commercial kit, albumin was measured. A spectrophotometer was used to measure the colour intensity for total protein and albumin at 546 and 570 nm, respectively. After deducting the albumin content from the total protein, the amount of globulin was calculated. Additionally, the albumin to globulin (A: G) ratio was determined.

(B) Muscle analysis

(I) Muscle water content: Sidwell *et al*., (1970) method for determining muscle water content was used.

(II) Muscle total protein: According to **Josyln (1950)**, the semi-microkjeldahl method was used to assess muscle total protein.

(III) Muscle total lipids : The common method described in **A.O.A.C. (1970)** was used to determine the total lipid content of fish muscle.

(IV) Muscle ash: The samples were burned for 16 hours at 650 ^{0}C in a muffle furnace to estimate the amount of muscle ash **(Sidwell et al., 1970)**.

(5) Statistical analysis

The results were statistically analysed using analysis of variance (F-test) followed by Duncan's multiple range test to determine differences in means using Statistical Analysis Systems, Version 6.2 **(SAS, 2000)**.

RESULTS AND DISCUSSION

Water pollution is a serious issue that affects humans, animals, and plants in a variety of harmful ways. Industrialization is causing this problem to grow, so it needs to be tracked down and looked into. Our study aims to evaluate the impact of copper in the form of copper sulphate (CuSO4), with or without curcumin diet supplementation (500 mg/kg diet) on some biological and physiological status of *Clarias gariepinus*.

Data concerning growth rates of the studied Nile catfish; *Clarias gariepinus* after exposure to different studied treatments for 12 weeks (Table 1) revealed that, fish fed commercial basal diet and exposed to sublethal concentration of copper (0.35 mg/l) exhibited lower growth rate (lower body weight gain, specific growth rate and a significant decrease of condition factor) compared to that of control group fish. Moreover, fish reared in dechlorinated tap water and fed 500 mg curcumin /kg ration showed highest growth parameters. However, fish exposed to 0.35 mg Cu/l and fed curcumin enriched basal diet revealed growth rates similar to that of control group.

The decrease in growth parameters of *Clarias gariepinus* may be attributed to copper accumulated in fish vital organs recorded in the present study. The same locating was previously made concerning fish that have been not able to thrive in deteriorated aquatic environments **(Haggag *et al*., 1999; Salah El-Deen *et al*., 1999; Zaghloul *et al*., 2006)**. Additionally, fish might need to detoxicate the metal or ameliorate the tempo at which the deactivated proteins are produced. Both options require more energy, which might be a reason for fish weight reduction. **Carvalho *et al*. (2004)** demonstrated a correlation between decreased fish weight and the activation of metal detoxing mechanisms, inclusive of an increase in metallothionein ranges.

According to **Bonga and Lock (1993)**, water-born toxicants have an effect on the gills via making the gill epithelium extra permeable to water and ions and via inhibiting the chloride cells' potential to trade ions. Reduced growth will take place from the fish's compensatory responses, which might dramatically boom the strength had to hold water and ion balance.

The present study revealed high copper concentrations in gills and liver of *Clarias gariepinus* than in muscles in case of copper treated group (Table 2). The liver is the primary organ in the body that detoxifies, transforms, and stores harmful substances, so, high propensity of copper to react with the oxygen carboxylate, amino group, nitrogen, and/or sulphur of the mercapto group in the metallothionein protein, whose concentration is highest in the liver, could explain the accumulation of metals in the liver **(Al-Yousuf *et al*., 2000)**. Because of its connection to low molecular weight proteins (such metallothionein) that are concentrated in hepatic tissues, the liver's increased copper accumulation as previously reported by **Zaghloul *et al*. (2006) and Abdel-Khalek *et al*. (2020)**. **Masoud *et al*.,(2007)**, reported high copper bioaccumulation in tissues may be attributed to direct contact of aquatic medium with gills, the metal concentrations in this organ correspond to those in the surrounding environment. Moreover, **Carvalho et al. (2004)** reported that copper toxicity is determined by the pH of the water. They also recorded an increase in hepatic metallothionein of a teleost (*Prochilodus scrofa*) exposed to copper at pH 8.0 than pH 4.5.

Table (1): Growth parameters of the Nile catfish; *Clarias gariepinus* exposed to sublethal concentration of copper and fed commercial basal diet or curcumin supplemented basal diet for 12 weeks.

Studied Groups	Body weight gain (g)			Specific growth rate (g/day)	Condition factor (k)
	Initial weight	Final weight	Weight Gain Percentage (%)		
Group I Control group (Fish reared in dechlorinated tap water and fed commercial basal diet)	51.17 ± 1.15 A	62.44 ± 0.91 B	**22.024**	0.278 ± 0.011 B	0.520 ± 0.01 A
Group II (Fish reared in dechlorinated tap water and fed curcumin supplemented basal diet, 500mg curcumin/kg diet)	50.83 ± 0.63 A	65.67 ± 0.49 A	**29.20**	0.357 ± 0.009 A	0.505 ± 0.012 A
Group III (Fish exposed to 0.35 mg Cu/l and fed commercial basal diet)	49.5 ± 1.14 A	51.17 ± 1.03 C	**3.37**	0.046 ± 0.003 C	0.397 ± 0.010 B
Group IV (Fish exposed to 0.35 mg Cu/l and fed curcumin supplemented basal diet, 500mg curcumin/kg diet)	52.5 ± 0.69 A	63.67 ± 0.62 A/B	**21.27**	0.268 ± 0.01 B	0.490 ± 0.007 A
F-values	**1.78**	**68.4****		**235.5****	**21.97****

Data are represented as means of eighteen fish ± S.E

Means within the same column, with the different letters for each parameter are significantly different (Duncan's multiple range test, SAS 2000).

** Highly significant difference ($P < 0.01$)

Table (2): Residual copper in some selected vital organs (mg / g dry weight) of the Nile catfish; *Clarias gariepinus* exposed to sublethal concentration of copper and fed commercial basal diet or curcumin supplemented basal diet for 12 weeks.

Studied Groups	Gills	Liver	Muscles
Group I Control group (Fish reared in dechlorinated tap water and fed commercial basal diet)	0.28 ± 0.03 B	2.13 ± 0.09 B	0.11 ± 0.02 B
Group II (Fish reared in dechlorinated tap water and fed curcumin supplemented basal diet, 500 mg curcumin/kg diet)	0.30 ± 0.04 B	2.23 ± 0.27 B	0.13 ± 0.04 B
Group III (Fish exposed to 0.35 mg Cu/l and fed commercial basal diet)	2.96 ± 0.13 A	7.74 ± 0.43 A	1.20 ± 0.10 A
Group IV (Fish exposed to 0.35 mg Cu/l and fed curcumin supplemented basal diet, 500 mg curcumin/kg diet)	0.39 ± 0.03 B	2.51 ± 0.16 B	0.15 ± 0.04 B
F-values	**317.8****	**99.3****	**66.9****

Data are represented as means of six samples ± S.E.
Means within the same column, with the different letters for each parameter are significantly different (Duncan's multiple range test, SAS 2000).
** Highly significant difference at $P < 0.01$

High metal concentrations in the gills may be caused by decreased pH levels at the gill surface brought on by CO2 respiration, which may breakdown metals and turn them into soluble forms that easily penetrate into the gill tissues, or by metals complexing with the mucus of gill lamellae **(Karadede-Akin and Unlu, 2007; Masoud *et al*., 2007).** However, there were significant levels of metals in the muscles, which may be related to the presence of fat in muscle tissues and their high propensity to mix with heavy metals **(Shenouda *et al*., 1992)**.

However, the low copper content in fish muscles in the present study, may be correlated with the fat-content in muscle tissues, low fat affinity to combine with heavy metals, and/or low metabolic activity of muscle. The recorded results are in agreement with **Uluturhan and Kucuksezgin (2007) and Tayel *et al*. (2008)**. In the present study, copper concentrations were higher in the gill than in the muscle tissue of the studied fish (*Clarias gariepinus*). Metal concentration in the gill could be due to copper complexion with the mucus that is impossible to completely remove from the lamellae, before tissue is prepared for analysis. Moreover, low levels of copper in the muscles may be attributed to the little blood supply in muscular tissues as previously reported by **Mohamed and Gad (2008)**.

Regarding blood parameters of *Clarias gariepinus* exposed to tenth half lethal concentration of copper and fed ration with or without curcumin are declared in Table 3. The present result revealed high significant decrease in RBCs, Hb and Ht values of fish exposed to tenth half lethal concentration of copper (0.35 mg/l), for 12 weeks in comparison to those of control group fish raised in dechlorinated tap water and fed commercial fish ration or supplemented with curcumin (5g/kg), may be attributed to reduction in red blood corpuscles production in the hematopoietic organs under the action of high water copper concentrations **(Zaghloul *et al.*, 2005, 2006 and 2016)** or may be oxidative damage of RBCs. Meanwhile, the present results revealed non-significant changes in the calculated blood derivatives, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of fish, among the different studied treatments.

In the current study, the significant increases of WBCs count could be attributed to an adaptation to the stressful effects of pollution. Another potential explanation for the elevated count of WBCs is the clearance of cell debris as a result of tissue injury as previously reported by **Hii *et al*. (2007)** in case of Asian swamp eels (*Monopterus albus*) exposed to water pollutants. The contaminants may change fish's immunological reactions because they have immunosuppressive or immunopotentiating effects **(Giron-Perez *et al*., 2008).** It is widely known that fish may have less nonspecific immunity if their leucocyte counts shift following exposure to contaminants. The regulation of immunological processes by leucocytes occurs in a wide range of organisms, and the increase in WBCs in stressed animals suggests a coping mechanism for stress **(Witeska, 2003)**.

Fish health biomarkers may be based on biochemical factors **(Tekmedash *et al*., 2016)**. Exposure of the studied fish fed on commercial basal diet to 0.35 mg copper/l for 12 weeks induced highly significant increase ($p < 0.01$) in the serum glucose levels than those of the control groups fish that raised in dechlorinated tap water and fed either commercial basal diet or curcumin enriched diet (5g/kg). The reason for elevation of glucose levels in blood of fish may be due to the high copper concentration in fish tissues, where it was proven that metals bioaccumulation changes carbohydrates metabolism, causing hyperglycaemia in fish **(Kumar *et al*., 2018)**. Moreover, **Öner *et al.* (2008)** demonstrated an increase in glucose levels in case of *Oreochromis niloticus* fish exposed to sublethal concentration of cadmium. **Banaee *et al.* (2019)** reported an increase in plasma glucose concentration in *Cyprinus carpio* after Cd exposure. Moreover, changes in the levels of blood glucose and total protein can be seen in case of liver failure **(Öner *et al*., 2008)**.

Table (3): Blood parameters of the Nile catfish; *Clarias gariepinus* exposed to sublethal concentration of copper and fed basal diet or curcumin supplemented basal diet for 12 weeks.

Studied Groups	RBCs ($x 10^6/mm^3$)	Hb (g/100 ml)	Ht (%)	MCV (μm^3/cell)	MCH (pg/cell)	MCHC (g/100 ml)	WBCs ($x 10^3/mm^3$)
Group I Control group (Fish reared in dechlorinated tap water and fed commercial basal diet)	2.60 ± 0.06 A	10.60 ± 0.13 A	33.67 ± 3.40 A	128.33 ± 10.3 A	41.0 ± 0.73 A	34.0 ± 3.60 A	29.33 ± 3.10 B
Group II (Fish reared in dechlorinated tap water and fed curcumin supplemented basal diet, 500mg curcumin/kg diet)	2.66 ± 0.11 A	10.37 ± 0.57 A	35.33 ± 1.12 A	118 ± 6.01 A	40.7 ± 0.92 A	35.0 ± 2.22 A	31.33 ± 2.74 B
Group III (Fish exposed to 0.35 mg Cu/l and fed commercial basal diet)	1.93 ± 0.08 B	7.53 ± 0.16 C	24.0 ± 1.31 B	136 ± 1.32 A	38.7 ± 1.12 A	29.33 ± 0.56 A	54.67 ± 4.20 A
Group IV (Fish exposed to 0.35 mg Cu/l and fed curcumin supplemented basal diet, 500mg curcumin/kg diet)	2.57 ± 0.19 A	9.27 ± 0.55 B	32.67 ± 2.35 A	129 ± 9.39 A	37.0 ± 2.53 A	33.67 ± 3.27 A	32.0 ± 3.14 B
F-values	8.04**	11.67**	3.57**	0.95	1.54	0.85	12.74**

Data are represented as means of six samples ± S.E.
Means within the same column, with the different letters for each parameter are significantly different (Duncan's multiple range test, SAS 2000).
** Highly significant difference at $P < 0.0$

In the metabolism and control of the water balance, total plasma proteins are crucial. The normal range of plasma total protein concentration in fish (4.08 ± 0.61g/dl) that reported by **Sabae and Mohamed, (2015)**. So up or down in the recorded plasma protein, could be useful in diagnosis of fish disease. The majority of plasma proteins that are produced by the liver are utilised as a measure of liver dysfunction **(Yang and Chen, 2003)**.

Many studies have reported change in blood glucose levels and protein profile of fishes in response to heavy metals **(Yacoub and Gad, 2012; Javed and Usmani, 2019)**. In the present study, there was elevation in serum total protein (hyperproteinaemia), albumin and globulin (Figs 1, 2 and 3) of *Clarias gariepinus* fed commercial basal diet and exposed to sublethal concentration of copper (0.35 mg/l) for twelve weeks in comparison with that of control group fish. The present findings are in agreement with previous reports of increased level of plasma proteins as a result of fish exposure to heavy metals **(Zaki *et al*., 2008)**. According to the current study, this may be caused by metabolic system activation in response to exposure to pollutants, cellular material degradation in the liver, various pathological conditions like liver and kidney damage, relative changes in the mobilization of blood proteins, water loss in the plasma, and/or induction of protein synthesis in the liver, which have all been previously reported by **Al-Attar (2005)**.

The observed increase in serum enzymes (AST, ALT and ALP) activities and kidney (creatinine and uric acid levels) functions (Table 4), may be attributed to copper bioaccumulation. Residual heavy metals in the liver and kidney caused malfunction and damage of the renal cells followed by an increase in serum creatinine and uric acid levels (**Haggag *et al*., 1999; Zaghloul., 2000**). Chang *et al*. (1996) concluded that kidney damage may result in reduced renal blood flow with reduction in glomerular filtration rate, resulting in azotemia characterized by increase in blood urea nitrogen, uric acid and creatinine.

The highly significant differences were obtained in serum protein profile (Figs 1, 2, 3 and 4) of *Clarias gariepinus* exposed to 0.35 mg/l for 12 weeks and fed commercial basal diet, with the highest value in serum protein (6.59 ± 0.21 g/dl), albumin (4.27 ± 0.06 g/dl), globulin (2.33 ± 0.21 g/dl) and A/G ratio than those of control groups fish that reared in dechlorinated tap water and fed either commercial basal diet or curcumin supplemented basal diet.

Blood protein levels typically fluctuate under many physiological and pathological circumstances **(Sabri *et al*., 2009)**. Under stressful circumstances, the plasma protein has undergone significant modification **(Gopal *et al*., 1997)**. Moreover, **Banaee *et al*. (2019)** noted a decrease in plasma total protein, albumin, and globulin in *Cyprinus carpio* after Cd exposure; Cd-exposed fish experienced changes in biochemical parameters and energy status.

The meat quality of fish exposed to 0.35 mg Cu/l and fed commercial basal diet for 12 weeks deteriorated in the present investigation (Table 5). There was considerable increase inside the muscle's water content and ash, in addition to considerable declines in the total protein and lipid content of the muscle. These findings corroborated the ones recognized by **Zaghloul (2001)** and **Elghobashy, *et al*. (2001)**, who recorded a decrease in muscle proteins and lipids of African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) subjected to high concentrations of heavy metals.

The high level of metal bioaccumulation in gills, recorded in the present study may be responsible for the decrease in total protein and total lipids in the muscles of fish exposed to sublethal concentration of copper. Copper bioaccumulation induce damage to the gill structure and a decrease in oxygen consumption, both of which result in a dramatic decrease in metabolic rate. Moreover, according to the current study's hyperglycaemia, which has a higher impact on proteogenic and lipogenic pathways, the drop in insulin level, which was a result of recorded hyperglycaemia, may also be responsible for the decrease in total muscle protein and total lipids **(Ablett, *et al.*, 1981)**. Additionally, the usage of body protein and/or fat as an energy source to satisfy the rising physiological demands was a direct cause of the decrease in body protein and lipids at inappropriate habitat.

Table (4): Glucose level, liver and kidney functions in serum of the Nile catfish; *Clarias gariepinus* exposed to sublethal concentration of copper and fed commercial basal diet or curcumin supplemented basal diet for 12 weeks.

Studied Groups	Glucose (mg/100 ml)	AST (u/l)	ALT (u/l)	ALP (u/l)	Creatinine (mg/100 ml)	Uric acid (mg/100 ml)
Group I Control group (Fish reared in dechlorinated tap water and fed commercial basal diet)	66.7 ± 3.39 B	22 ± 2.39 B	8.0 ± 0.37 B	16.7 ± 0.84 B	1.50 ± 0.14 B	12.50 ± 0.86 B
Group II (Fish reared in dechlorinated tap water and fed curcumin supplemented basal diet, 500 mg curcumin/kg diet)	64.0 ± 4.21 B	21.0 ± 0.73 B	7.0 ± 0.09 B	17.0 ± 1.6 B	1.30 ± 0.17 B	14.67 ± 1.48 B
Group III (Fish exposed to 0.35 mg Cu/l and fed commercial basal diet)	98.3 ± 4.04 A	42.67 ± 2.49 A	17.0 ± 0.73 A	24.3 ± 1.84 A	4.13 ± 0.09 A	31.0 ± 2.63 A
Group IV (Fish exposed to 0.35 mg Cu/l and fed curcumin supplemented basal diet, 500mg curcumin/kg diet)	68.0 ± 0.73 B	24 ± 2.0 B	8.3 ± 1.32 B	19.3 ± 1.60 B	1.73 ± 0.09 B	16.0 ± 0.97 B
F-values	29.17**	25.32**	26.4**	5.37**	105.7**	26.4**

Data are represented as means of six samples ± S.E.
Means within the same column, with the different letters for each parameter are significantly different (Duncan's multiple range test, SAS 2000).
** Highly significant difference at P < 0.01.

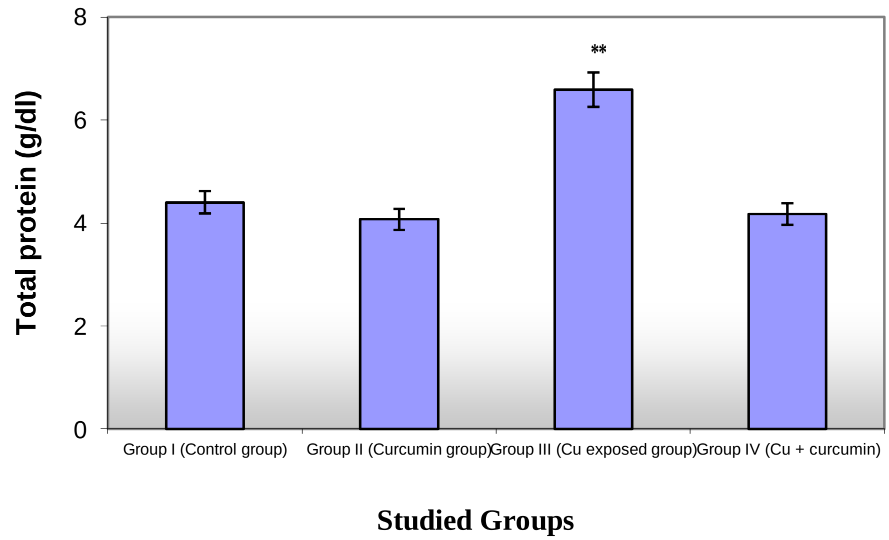


Fig.(1): Serum total protein of *Clarias gariepinus* exposed to 0.35 mg Cu/l and fed ommercial basal diet or curcumin supplemented diet (500 mg/Kg diet) for 12 weeks. ** Highly Significant difference at P < 0.01.

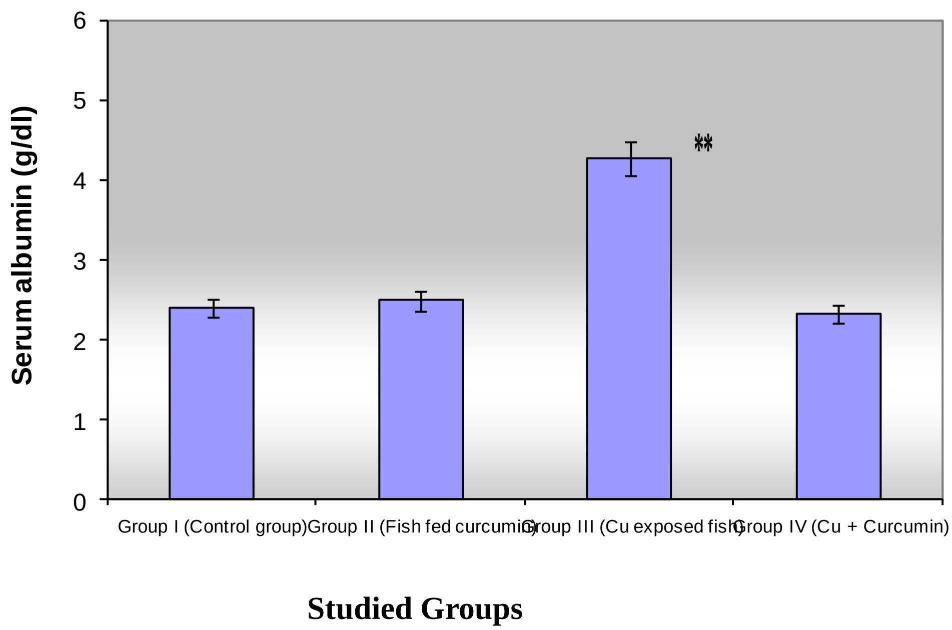
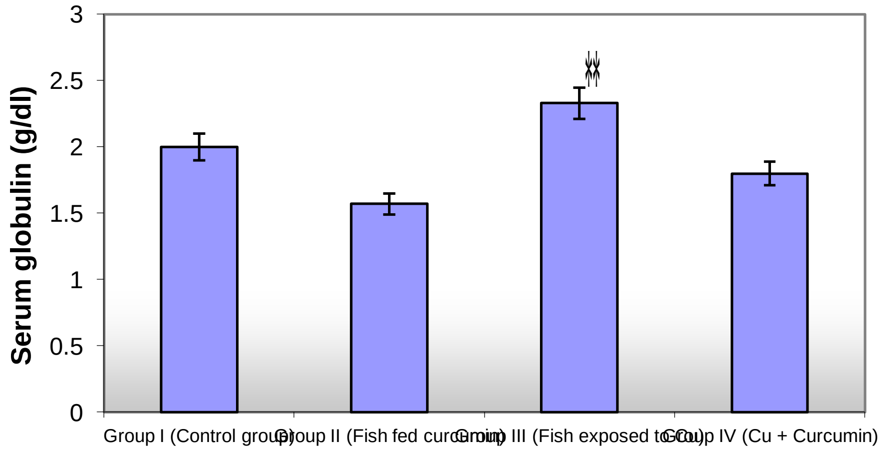
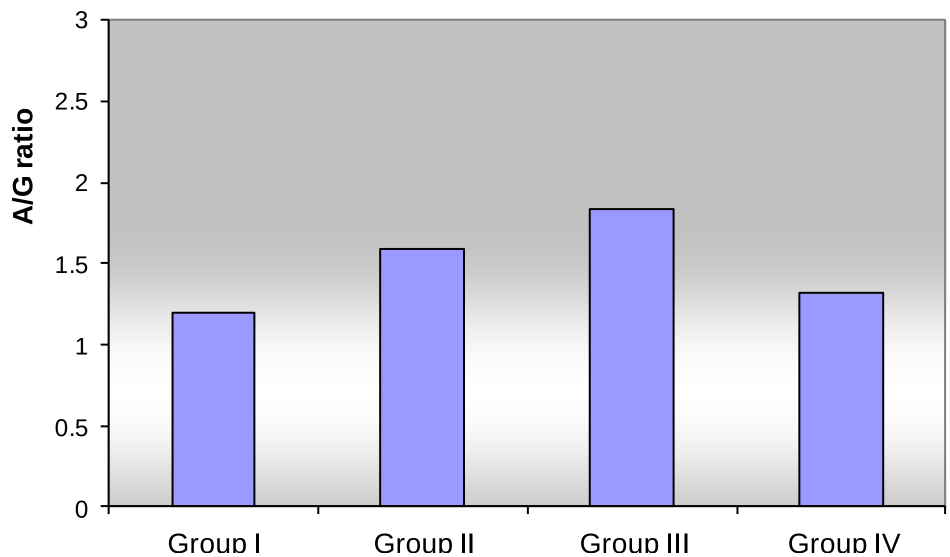


Fig.(2): Serum albumin of *Clarias gariepinus* exposed to 0.35 mg Cu/l and fed commercial basal diet or curcumin supplemented diet (500 mg/Kg diet) for 12 weeks. ** Highly Significant difference at P < 0.01



Studied Groups

Fig.(3): Serum globulin of *Clarias gariepinus* exposed to 0.35 mg Cu/l and fed commercial basal diet or curcumin supplemented diet (500 mg/Kg diet) for 12 weeks. ** Highly Significant difference at $P < 0.01$



Studied groups

Fig.(4): Serum Albumin/Globulin ratio of *Clarias gariepinus* exposed to 0.35 mg Cu/l and fed basal diet or curcumin supplemented basal diet (500 mg/kg).

Increasing the supplementary dietary curcumin to 500 mg/kg diet restore the muscle quality and other studied parameters of fish exposed to the same copper concentration to that of control group fish. In the present study, curcumin was used as antidote against the copper induced toxicity in *Clarias gariepinus*. The studied biological, haematological, as well as biochemical parameters of *Clarias gariepinus* fish exposed to 0.35 mg Cu/l and fed fish ration supplemented with curcumin (5g/kg), restored more or less similar to normal as that of control groups fish raised in dechlorinated tap and fed commercial fish ration or supplemented with curcumin (5g/kg). This is in agreement with the results of Mise et al. (2017) and Xu et al. (2018) who reported that defence mechanism in the fish has enhanced many folds due to the defence activity of curcumin. Curcumin possesses dual antioxidant activity and acts through the scavenging reactive oxygen species (ROS) including superoxide and hydroxyl radicals due to its phenolic structure, and induces the upregulation of several endogenous cytoprotective and antioxidant proteins. It is also an effective lipid peroxidation inhibitor (Kumari and Paul, 2020).

Table (5): Meat quality of the Nile catfish; *Clarias gariepinus* exposed to sublethal concentration of copper and fed commercial basal diet or curcumin supplemented basal diet for 12 weeks.

Studied Groups	Water contents (%)	Total protein (% of wet weight)	Total lipids (% of wet weight)	Ash (%)
Group I Control group (Fish reared in dechlorinated tap water and fed commercial basal diet)	75.3 ± 0.56 B	17.0 ± 0.37 A	4.3 ± 0.2 A	1.77 ± 0.06 B
Group II (Fish reared in dechlorinated tap water and fed curcumin supplemented basal diet, 500mg curcumin/kg diet)	74.9 ± 0.37 B	18.0 ± 0.36 A	4.33 ± 0.21 A	1.87 ± 0.10 B
Group III (Fish exposed to 0.35 mg Cu/l and fed commercial basal diet)	82.70 ± 0.55 A	12.33 ± 0.28 B	2.47 ± 0.20 B	3.13 ± 0.076 A
Group IV (Fish exposed to 0.35 mg Cu/l and fed curcumin supplemented basal diet, 500mg curcumin/kg diet)	76.66 ± 0.80 B	16.33 ± 0.55 A	3.87 ± 0.08 A	2.06 ± 0.14 B
F-values	38.3**	37.7**	23.6**	50.9**

Data are represented as means of six samples ± S.E.

Means within the same column, with the different letters for each parameter are significantly different (Duncan's multiple range test, SAS 2000).

** Highly Significant difference at $P < 0.01$.

CONCLUSION

In light of the fact that communities rely heavily on fish as a major source of protein, one could draw the conclusion that the degradation of aquatic habitats with high levels of heavy metals, particularly copper, bioaccumulated in one of the most common wild and cultured fish species, *Clarias gariepinus*, gives us cause for concern about community health issues. Additionally, including curcumin in the fish diet as an antioxidant prevents copper poisoning , returns the physiological status to normal, and enhances the quality of flesh.

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