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BIOCHEMICAL AND HAEMATOLOGICAL CHANGES IN TILAPIA NILOTICA (OREOCHROMIS NILOTICUS) EXPOSED TO COPPER

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SUMMARY

The effect of exposure to sublethal concentration of copper sulfate for 3 weeks was studied in *Tilapia nilotica* fish. Marked elevations were recorded in the activities of serum glutamic pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP). Serum total protein was increased with hyperglycaemia, while cholesterol level was reduced. Copper induced polycythemia accompanied by blood haemoglobin (Hb) level, haematocrit (Hct) value, and mean corpuscular haemoglobin concentration (MCHC). In addition, leucopenia, lymphopenia and neutrophilia were observed. It was concluded that copper produces tissue damage, metabolic stress and malfunctions of the haematopoietic system in *Tilapia nilotica.*

INTRODUCTION

Copper is an essential trace element for many biological processes. However, at high concentrations it is highly toxic (Tort et al., 1987). The use of copper in industry and agriculture polluts natural water and may cause significant tissue damage in fish (McKim et al., 1970). This tissue damage can be demonstrated by the changes in activity of the cellular enzymes in sera. The increase in activity of these enzymes depends primarily on the magnitude and severity of cell damage (Kristoffersson et al., 1974; Nemcsok and Boross, 1982; Asztalos and Nemcsok, 1985; Asztalos et al., 1990). The rise in blood glucose level has been used for detection of metabolic stress (Nemcsok and Boross, 1982). In addition, haematology is used as an index of the health status in fish (Blaxhall, 1972) due to the close association between the circulatory system of fish and the external environment (Casillas and Smith, 1977). The blood parameters of diagnostic importance such as erythrocyte and leucocyte counts, haemoglobin concentration, haematocrit value and leucocytic differential counts usually readily respond to incidental factors such as physical and environmental stress due to water contaminants (Buckley, 1977; Railo et al., 1985).

The present work was undertaken to study the effect of sublethal concentration of copper on some biochemical and haematological parameters of *Tilapia nilotica.* Fish were selected because of their wide availability, edibility in Egypt, and their important ecological role in the Nile River.

MATERIAL AND METHODS

Fifty *Tilapia nilotica (Oreochromis niloticus)* weighing from 120 to 160 grams were obtained from the Nile river at Giza City. Fish were maintained in tanks containing well-areated water at atmospheric temperature for one week before

the experiment. They were fed a commercial fish diet at a rate of 1% live weight per day during the acclimation period as well as during treatment (Riva and Flos, 1993). Fish were divided into 2 groups (n=25 individual each), the first was exposed to copper sulphate (El-Nasr Pharmaceutical Chemical Co.) level of 1 mg/1 (Gardner and LaRoche, 1973) while the other one served as control. Blood samples were collected from the caudal vein after 1, 3, 7, 14 and 21 days of exposure. Part of blood was left to clot and then centrifuged at 3000 r.p.m. to obtain serum for biochemical studies. The other part was heparinized for haematological investigations.

Biochemical studies

Test kits of Bio Merieux (France) were used for determination of the activity of serum glutamic pyruvic transaminase, GPT, and glutamic oxalacetic transaminase, GOT (Reitman and Frankel, 1957), lactate dehydrogenase, LDH, (Annon, 1971) and alkaline phosphatase, ALP, (Bessy, 1946). Serum glucose was assessed by (Trinder 1969). The method of Flegg (1973) was adopted to assess serum cholesterol and the Biuret reaction was followed to determine serum total protein level (Weichselbaum, 1946).

Haematological investigations

Blood haemoglobin (Hb) was assessed by cyanmethaemoglobin method (Drabkin, 1946). The other parameters were determined according to Stoskopf (1993). Red blood cell (RBC) and white blood cell (WBC) counts were determined by haemocytometer. Blood smears were stained with Giemsa stain for differential leucocytic counts. Haematocrit value was carried out by using microhaematocrit capillary tubes centrifuged at 12000 r.p.m. for 5 min. The mean corpuscular haemoglobin concentration was then calculated.

Statistical analysis

The obtained data were subjected to the Stude t-test (Gad and Weil, 1986).

RESULTS

Biochemical studies

Table 1 presents the changes of some biochemi constituents in blood of *T. nilotica* due to copp sulphate exposure. The obtained data revealed th serum GPT activity increased significantly after 7, 14 days ($P < 0.01$) and 21 days ($P < 0.0$ Significant elevation in serum GOT activity w also observed on the 3rd, 7th, 14th day ($P < 0.0$ and on the 1st and 21st day of experime ($P<0.05$). LDH serum activity was elevated alon the whole period of experiment ($P < 0.01$). Seru ALP activity rose on the 21st day ($P<0.01$) an the 3rd and 14th day ($P < 0.05$).

Hyperglycaemia was a constant finding from th beginning until the end of the experimen ($P<0.01$). Serum total protein level showed significant increase after 7 and 14 days ($P<0.05$) while serum cholesterol concentration wa reduced on the 14th and 21st day ($P<0.01$) and o the 7th day ($P<0.05$).

Haematological studies

The effect of copper sulfate exposure on RBC count, Hb level, Hct value and MCHC was recorded in Table 2. The total and differential leucocytic counts were shown in Table 3.

Polycythemia was observed on the 21st day ($P<0.01$) and the 3rd, 7th and 14th day ($P<0.05$). Blood Hb concentration rose significantly on the 7th and 21stday ($P<0.01$) and on the 3rd and 14th day ($P<0.05$). The Hct value showed significant increases ater 3 days ($P<0.01$) and after 1 and 21

Table 1: Effect of copper sulfate exposure on serum biochemical profile of *T. nilotica*

Parameter	Exposure period (days)									
	1		3		7		14		21	
	C	E	C	E	C	E	C	E	C	E
GPT (I.U/l)	42.13 ± 2.34	48.82 ± 2.38	43.70 ± 2.30	66.38** ± 2.16	44.23 ± 2.09	84.41** ± 2.55	50.83 ± 2.37	68.22** ± 2.68	47.68 ± 2.11	55.22* ± 2.32
GOT (I.U/l)	50.52 ± 3.20	66.20* ± 3.67	51.29 ± 3.06	85.48** ± 3.91	57.91 ± 3.39	83.91** ± 3.71	49.29 ± 2.73	70.76** ± 3.46	50.44 ± 3.03	61.28* ± 2.88
LDH (I.U/l)	204.56 ± 4.71	310.94** ± 5.35	220.20 ± 4.10	387.25** ± 5.29	211.25 ± 3.82	369.38** ± 5.69	202.44 ± 4.44	314.26** ± 5.34	192.46 ± 4.72	270.60** ± 5.87
ALP (K.AU/l)	4.16 ± 1.55	6.82 ± 1.49	5.42 ± 1.47	10.97* ± 1.70	5.08 ± 1.58	8.28 ± 1.86	4.84 ± 1.93	12.36* ± 1.97	3.29 ± 1.50	15.42** ± 2.06
Glucose (mg/dl)	41.20 ± 2.54	120.62** ± 2.85	45.64 ± 2.33	145.84** ± 3.96	46.90 ± 2.51	169.29** ± 3.58	42.34 ± 2.69	114.59** ± 3.30	48.41 ± 3.56	102.81** ± 3.79
Protein (g/dl)	2.34 ± 0.22	2.48 ± 0.41	2.41 ± 0.44	2.74 ± 0.42	2.46 ± 0.37	3.84* ± 0.42	2.36 ± 0.28	3.49* ± 0.30	2.43 ± 0.32	3.18 ± 0.35
Cholesterol (mg/dl)	146.26 ± 4.15	152.38 ± 4.41	137.54 ± 4.02	140.82 ± 3.79	134.53 ± 3.90	120.18* ± 3.44	144.20 ± 4.46	94.82** ± 3.50	158.74 ± 3.91	108.77** ± 4.02

Values are expressed as Mean ± SE, C = control, E = experimental
n = 5, * $P < 0.05$, ** $P < 0.01$

Table 2: Effect of copper sulfate exposure on RBC count, Hb level, Hct value and MCHC in *T. nilotica*

Parameter	Exposure period (days)									
	1		3		7		14		21	
	C	E	C	E	C	E	C	E	C	E
RBC $10^6/mm^3$	2.15 ± 0.10	2.17 ± 0.26	2.18 ± 0.22	3.24* ± 0.35	2.21 ± 0.14	3.12* ± 0.30	2.14 ± 0.24	2.93* ± 0.22	2.02 ± 0.14	3.43** ± 0.37
Hb g/dl	9.56 ± 0.65	10.74 ± 1.27	9.62 ± 0.69	13.03* ± 1.09	9.71 ± 0.39	14.40** ± 0.97	9.65 ± 1.05	12.97* ± 0.83	9.66 ± 0.92	14.20** ± 0.79
Hct %	24.80 ± 1.76	29.80* ± 1.22	25.2 ± 1.33	33.00** ± 1.54	22.60 ± 1.52	28.60 ± 1.86	22.40 ± 1.96	27.60 ± 1.45	24.20 ± 1.27	29.80* ± 1.48
MCHC %	38.52 ± 1.51	36.04 ± 1.45	38.17 ± 1.35	39.48 ± 1.68	42.96 ± 1.99	50.35* ± 1.91	43.08 ± 1.48	46.99 ± 1.54	39.92 ± 1.61	47.65* ± 1.68

Values are expressed as Mean ± SE, C = control, E = experimental n = 5, * P < 0.05, ** P < 0.01

Table 3: Effect of copper sulfate exposure on total and differential leucocytic count in *T. nilotica*

Parameter	Exposure period (days)									
	1		3		7		14		21	
	C	E	C	E	C	E	C	E	C	E
WBC $10^4/mm^3$	6.51 ± 0.86	4.28* ± 0.35	6.54 ± 0.42	4.32* ± 0.76	6.52 ± 0.54	4.451* ± 0.60	6.48 ± 0.87	5.10 ± 0.82	6.41 ± 0.94	6.21 ± 0.88
Lymphocyte %	59.20 ± 1.48	47.60** ± 1.36	61.60 ± 1.35	52.40** ± 1.88	61.80 ± 1.85	52.80* ± 1.69	68.20 ± 1.78	58.00 ± 1.39	62.00 ± 1.63	55.80* ± 1.79
Neutrophils %	23.80 ± 1.48	28.40 ± 1.69	22.60 ± 1.46	27.20 ± 1.49	21.40 ± 1.38	28.40** ± 1.47	21.20 ± 0.85	26.00* ± 1.62	22.80 ± 1.13	30.60** ± 1.66
Monocytes %	2.00 ± 0.32	3.60 ± 1.26	2.80 ± 0.58	3.20 ± 0.37	1.40 ± 0.51	2.40 ± 0.89	0.20 ± 0.2	1.00 ± 032	0.00 ± 0.00	1.20 ± 0.37
Eosinophils %	8.60 ± 1.09	12.10 ± 1.29	7.40 ± 1.65	10.80 ± 0.80	7.80 ± 1.06	8.80 ± 0.95	9.20 ± 1.22	8.60 ± 1.51	8.40 ± 0.68	7.60 ± 1.62
Basophils %	6.40 ± 0.84	8.00 ± 0.81	5.60 ± 0.90	7.40 ± 0.56	7.60 ± 0.53	6.60 ± 0.85	6.20 ± 0.98	5.80 ± 0.60	6.80 ± 0.71	6.00 ± 0.68

Values are expressed as Mean ± SE, C = control, E = experimental n = 5, * P < 0.05, ** P < 0.01

days ($P<0.05$). Significant elevations were recorded in the MCHC after 7 and 21 days ($P<0.05$).

Leucopenia was observed from the beginning until the 7th day of experiment at $P<0.05$. Lymphocytopenia was recorded after 1 and 3 days ($P<0.01$) and after 7 and 21 days ($P<0.05$). A significant neutrophilia was observed after 7 and 21 days ($P<0.01$) and after 14 days ($P<0.05$).

DISCUSSION

Exposure of *Tilapia nilotica* to sublethal concentration (1 mg/l) of copper sulfate for 3 weeks resulted in marked changes in the activities of serum GPT, GOT, LDH and ALP. The present findings agree with those of Vig et al. (1987) and Asztalos et al. (1990) who recorded increases in serum transaminases activities in copper-exposed carp (*Cyprinus carpio*). However, Mckim et al. (1970) reported that the exposure to copper increased then decreased GOT activity in brook trout (*Salvelinus fontinalis*). The elevation in transaminases activities may be attributed to liver injury (Bell, 1968; Kristoffersson et al., 1974). The marked rise in serum LDH activity recorded in the present work is in agreement with the results of Asztalos et al. (1990) in *Cyprinus carpio* due to copper exposure. This can be attributed to the damaging effect of copper on heart, liver and/or skeletal muscle (Asztalos and Nemcsok, 1985).

The hyperglycaemia observed in the present investigation is similar to that obtained in rainbow trout, *Salmo gairdneri,* (Lauren and McDonald, 1985), *Cyprinus carpio* (Vig et al., 1987) and freshwater teleost, *Labeo rohita,* (Radhakrishaniah et al., 1992). Unlike our results, Lanno et al. (1985) reported that exposure to copper had no significant effect on blood glucose in *Salmo gairdneri.* The blood glucose level reflects the changes in carbohydrate metabolism under hypoxia and stress conditions (Soivio et al., 1974). Rise of glucose level indicates the presence of stressful stimuli eliciting rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue (Mazeaud et al., 1977). Moreover, Shaffi (1979) attributed the hyperglycaemia in fish during pesticide poisoning to activation of phosphorylase enzyme system.

Concerning serum protein level, significant increase was noted in copper exposed fish. Our results confirmed the findings of McKim et al. (1970) who obtained similar results in *Salvelinus fontinalis.* Contrary to our results, O'Neill (1981) recorded reduction of protein level in *Cyprinus carpio* exposed to copper. The elevated protein concentration may be due to the induction of protein synthesis in the liver (Hilmy et al., 1979). Serum cholesterol level showed significant decrease in *T. nilotica* exposed to copper. Our results are in partial agreement with those of Munoz et al. (1991) who recorded rise followed by decrease in blood cholesterol concentration in *Salmo gairdneri.* Reduction of blood cholesterol may be due to greater level of utilization of cholesterol during corticosteroidogenesis, as it is a precursor for steroid hormones, or depressed *de novo* synthesis (Ferrando and Andreu-Moliner, 1991). In addition, Dutta and Haghighi (1986) reported that the rise in blood protein resulted in high density lipoprotein in serum and was suggested to be the cause of hypocholesterolemia in mercury-exposed bluegill (*Lepomis machrochirus*).

Regarding the impact of copper on the haematological profile of *T. nilotica,* polycythemia accompanied by elevated Hb level, Hct value and MCHC were observed. Similar to our findings, elevated RBC count, Hb level and Hct value were noted in copper-exposed *Salvelinus fontinalis* (McKim et al., 1970). Also Christensen et al. (1972) recorded an increase in

Hb and Hct in the brown bullhead, *Ictalurus nebulossus,* exposed to copper, but the RBC count was constant. Cyriac et al. (1989) observed raised Hct value in *Oreochromis mossambicus* but unlike our results Hb level was reduced. Gill et al. (1991) recorded polycythemia in rosy barb (*Barbus conchonius)* but contrary to our findings Hb level and MCHC were lowered. On the contrary, erythropenia with reduced Hb concentration and Hct value were recorded in *Clarias lazera* exposed to copper (El-Domiaty, 1987).

The increased RBC count may be due to stimulation of erythropoiesis by elevated demands for O_2 or CO_2 transport as a result of increased metabolic activity or by destruction of gill membrances causing faulty gaseous exchange (Buckley, 1977). It was found that copper penetrates the intact RBC inhibiting glycolysis, denaturing Hb and oxidising glutathione (Fairbanks, 1967). The increased Hb content could thus be explained as a process where the body tries to replace the oxidized or denatured Hb (Cyriac et al., 1989). The increases observed in Hct value and MCHC may be attributed to the polycythemia and elevated blood Hb level, respectively. The increased Hct value can also be attributed to swelling of RBCs due to increased PCO_2 in blood, hypoxia or stressful procedures (Soivio et al., 1974).

Responses of WBC to copper poisoning in *Tilapia nilotica* included leucopenia with lymphopenia and neutrophilia. These results confirmed the findings of Dick and Dixon (1985) who reported that acute exposure of *Salmo gairdneri* to copper induced leucopenia, the majority of which is the results of lymphopenia, whereas, chronic exposure to the same metal resulted in significant neutrophilia. Also Gill et al. (1991) observed lymphopenia after 2 weeks of exposure of *Barbus conchonius* to copper, but unlike our findings there was a significant increase in monocyte and basophil counts. The observed leucopenia can be attributed to generalized stress response causing increased pituitary-interrenal activity (Donaldson and Dye, 1975; Donaldson, 1981) resulting in increased secretion of corticosteroids (Ellis, 1981). The leucopenia could further have been aggravated by the necrosis of the leucopoietic tissue (Wepener et al., 1992). On the basis of the present study, it seems reasonable to assume that copper induced tissue damage as indicated by significant elevation in the studied enzymes, metabolic stress (hyperglycaemia) and malfunctions of the haematopoietic system in intoxicated fish.

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