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Hematological and serum biochemical studies in fresh water fish exposed to acute and chronic copper and mercury toxicity

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A total number of 668 apparently healthy fish were obtained from farm to study the effect of two heavy metals in a form of (Copper sulfate and Mercuric chloride) on some hematological and biochemical parameters of blood. The LC_{50} /96 hr. of Cu and Hg were estimated and fish exposed to ½ LC_{50} for 7 days and for $1/_{10}$ LC_{50} for 8 weeks from each product separately. Results showed decrease in RBCs count, PCV% and Hb in acute and chronic mercury while a significant increase was shown in acute and chronic copper toxicity, total leucocytic count showed decrease in acute mercury toxicity and increase in the chronic case, while in copper toxicity non-significant decrease in acute and significant decrease in chronic toxicity was noticed. Elevated serum urea and creatinine in both acute and chronic mercury and copper toxicity was detected. No changes in total bilirubin in the acute mercury and chronic copper toxicity while significant increase in chronic mercury and acute copper. Elevation of serum AST and ALT in some days of acute toxicity of mercury and copper while in chronic mercury toxicity a significant elevation of both serums AST and ALT were detected .while in chronic copper toxicity serum AST was fluctuated and ALT showed no significant changes. CK study revealed significant decrease in acute mercury with fluctuation in the chronic toxicity while in copper toxicity it showed fluctuation in acute and significant decrease in chronic toxicity. Glucose value decreased in acute and chronic mercury toxicity while in copper toxicity it showed significant increase in the acute and increase followed by significant decrease in the chronic copper toxicity.

Key words: Immunology, Toxicity, Copper, Mercury, Residue, Fish

Mercury is a natural element that cannot be created or destroyed. It can combine chemically with other materials to create organic (carbon-containing) and inorganic (not containing carbon) compounds *USEPA (2007*). Natural and human (anthropogenic) activities can redistribute this element in the atmospheric, soil and water ecosystem through a complex combination of transport and transformations. *(Pirrone et al., 2000, Wängberg et al., 2001 and Munthe et al., 2001).*

Mercury has a wide variety of other uses in military application, batteries, medicine, electrical equipment, paint and wood pulping industries. Mercury is emitted to the atmosphere from a variety of natural and anthropogenic sources as volcanic emissions, wind-blown dusts, from small scale fuel burning, electric lamp breakage, laboratory use, dental preparation, landfills, sludge application, combustion processes, manufacturing industries and wide range of minor sources. *(Pacyana et al., 2000).*

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Copper is emitted into the air naturally from wind-blown dust, volcanoes and anthropogenic sources, the largest of which primary copper smelters and/ or processing facilities *(Romo-Kroger et al., 1994).*

Copper is an essential element in human and animals. Most of the adverse health effects associated with copper result of its deficiency. Efficient homeostatic mechanisms generally protect mammals from the adverse effect of copper excess *USEPA (1987).*

copper is a component of enzymes that are vital in hematopoiesis, maintenance of vascular and cellular integrity and structure and function of central nervous system *(O'Dell 1976).*

The intents of the present study were directed toward evaluation of the effects of mercury and copper as heavy metals pollutants on the fresh water fish Nile tilapia through studying the Clinical and biochemical changes of polluted Nile tilapia after exposure to short (acute) and long term (chronic) toxicity of copper and mercury separately.

MATERIALS AND METHODS

Six hundred and sixty eight apparently healthy Nile tilapia fish (Oreochromis niloticus) of both sexes, weighing 130-170 g. and of 20-25cm length were used. They were gathered from El-Wafa farm, Manyal Sheha, Giza Province. During the transportation from the location to the laboratory, fish were kept in big plastic containers filled with the water from same water source and spilled with air by portable aerator to supply fish with needed air.

Heavy metals

Each of mercuric chloride ($HgCl_2$, MW 271.50 provided by Rhone-Poulenc-Paris, France) and copper sulfate ($CuSo_4$; MW 249.68 provided by El-Nasr-Adwic Company-Egypt) were added to aquarium's water in different concentrations of LC_{50} / 96 hr during short term and long term exposure experiments.

LC50 / 96hr of mercuric chloride in Nile tilapia were found to be 0.8 mg /L and for copper sulfate 0.759 mg /L.

Aquaria

Ten glass aquariums sized 100x30x50 cm with density of 16 fish for each. Supported by air water machines and filled with dechlorinated water were used for fish existing.

Fish were acclimatized to water and kept under observation in those aquariums for two weeks before the start of the experiment.

The water containing heavy metals for all aquaria used during the experiments was syphoned and replaced daily by water containing the heavy to guarantee the concentration of heavy metal in the experiment.

Three blood samples were aspirated (blood of four fish was pooled to constitute one sample) from the caudal vein according to the practical method of *Andrew (1990).*

The collected blood samples were used as follows: Sample collected on EDTA for haemogram estimation. Samples without anticoagulant for serum separation.

Haemogram estimation: Blood samples collected in dry clean tubes containing EDTA were subjected to RBCs count, WBCs count, Hb concentration, PCV % and differential leukocytic count. Blood indices (M.C.V, M.C.H. and M.C.H.C) were calculated from the obtained values of RBCs, PCV and Hb according to *Feldman et al. (2000).*

Serum biochemical analyses: Urea value was analysed according to *Patton and Crouch (1977).* Creatinine value was measured by photometric way according to *Henry (1974).* Bilirubin value was estimated according to *Jendrassik and Grof(1938).* Serum AST activity was determined by the method of *(Reitman and Frankel (1957).* Serum ALT activity was determined by the method of *(Reitman and Frankel (1957).* Serum CK was determined by the method mentioned by *(Stein 1998).*Glucose was determined after Thomas (1998).

RESULTS

Short term exposure to sublethal dose ($^1/_2$ LC_{50}/ 96 hr.) of mercuric chloride in Nile tilapia

Erythrogram Erythrocytes (RBCs) count, Hb content and PCV % were significantly decreased along the exposure time. Mean corpuscular volume (MCV) showed significant increase only in the 2nd day of exposure then gradually decreased to a significant level at the 7th day. A significant decrease was recorded for MCH and MCHC at the 5th and the 7th day of exposure. Calculation of RBCs indices indicated that the anemia was macrocytic normochromic at the beginning of the experiment then progressed to be microcytic hypochromic type at the end of the experiment (Table1). Total leucocytic count and heterophil's count showed significant decrease along the whole intervals of the experiment. Significant absolute lymphopenia especially on the 5th and the 7th day of exposure and monocytopenia appeared on the 7th day of exposure. This was accompanied with normal eosinophilic and basophilic counts (Table 2).

The determined results revealed significant elevation of urea and creatinine along the experimental period. Total Bilirubin Results obtained showed no significant change in the value of total bilirubin along the whole period of the experiment. There was significant elevation of serum AST activity started from the 5th day of exposure until the end of the experiment. Serum ALT activity was elevated only on the 2nd day

Table 1. Erythrogram of Nile tilapia fish exposed to mercuric chloride ½ LC_{50} / 96 hr for 7 days

Time (days)	Group	RBCs (10^6/μl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
2	C	1.58±0.17	6.85±0.72	17.25±1.26	109.60±3.86	43.43±2.81	39.63±2.07
	T	1.09*±0.09	4.95*±0.51	14.00*±0.82	128.75*±1.71	45.75±6.69	35.53±5.07
5	C	1.63±0.09	7.13±0.51	17.25±0.96	106.65±11.74	44.28±2.73	41.37±3.30
	T	1.29*±0.09	4.45*±0.53	14.00*±0.82	108.10±4.48	34.49*±5.38	31.88*±3.84
7	C	1.63±0.22	6.73±0.54	16.75±0.96	107.43±8.75	43.47±4.37	40.06±2.66
	T	1.25*±0.13	3.00*±0.50	11.00*±2.00	87.79*±7.75	24.32*±4.73	27.76*±4.92

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

Table 2. Leukogram of Nile tilapia fish exposed to mercuric chloride ½ $LC_{50.}$ / 96hr for 7 days

Time (days)	Group	WBcs (10^3/μl)	Differential Leucocytic count				
			Heterophils (10^3/μl)	Lymphocytes (10^3/μl)	Monocytes (10^3/μl)	Eosinophils (10^3/μl)	Basophiles (10^3/μl)
2	C	8.25±0.96	1.64±1.38	6.52±1.35	0.09±0.16	0	0
	T	5.25*±1.26	0.91*±1.30	4.25±1.47	0.08±0.13	0	0
5	C	8.75±0.96	1.71±0.75	6.94±0.67	0.10±0.26	0	0
	T	6.75*±1.26	1.14*±0.75	5.51*±0.77	0.10±0.13	0	0.003±0.1
7	C	9.00±0.82	1.84±0.57	7.06±0.67	0.10±0.17	0	0
	T	5.00*±0.82	0.84*±0.42	4.08*±0.53	0.07*±0.12	0	0

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

of exposure then returned to normal until the end of the experiment .There was significant decrease in serum CK activity started from the beginning of exposure until the end of the experiment .No change in the serum glucose level was detected on the 2nd day of exposure. Significant hypoglycemia was detected from the 5th day of exposure to the end of the experiment (Table 3).

Long-term exposure to sublethal dose ($^1/_{10}$ LC_{50} /96 hr.) of mercuric chloride in Nile tilapia fish.

Red blood cells (RBCs), Hb content and PCV revealed significant decrease than normal starting from the 2nd week of exposure till the end of the experiment. Value of MCV showed significant increase in the 6th and 8th week of exposure while MCH showed non-significant decrease along the whole period of exposure. Significant decrease was noticed in MCHC on the 6th and 8th week of exposure. Calculation of RBCs indices indicated that the anemia was normocytic normochromic at the first month of the experiment which changed to be macrocytic hypochromic at the second month of the experiment (Table 4).

Total leucocytic and heterophils, counts showed significant increase from the 2nd week to the end of

Table 3. Some serum biochemical constituents of Nile tilapia fish exposed to ½ LC_{50}/96 hr. of mercuric chloride for 7 days

Time (days)	Group	Blood urea (mg/dl)	Creatinine (mg/dl)	Total billirubin (mg/dl)	AST (U/L)	ALT (U/L)	CK (U/L)	Glucose (mg/dl)
2	C	10.66±0.55	0.95±0.07	0.61±0.12	17.50±3.87	19.00±2.31	3035±3.14	91.08±4.21
	T	14.05*±0.51	1.55*±0.11	1.09±0.48	21.00±4.40	30.25*±8.77	1415*±6.37	86.19±6.38
5	C	10.26±0.92	0.92±0.04	0.69±0.14	21.50±2.08	18.50±1.91	2899±5.66	93.20±1.14
	T	13.47*±0.60	1.42*±0.16	0.49±0.32	48.75*±19.33	22.00±5.03	1024*±3.59	68.67*5.33
7	C	9.99±0.76	0.90±0.04	0.59±0.20	20.75±2.75	17.50±2.50	2999±3.00	91.02±3.90
	T	13.30*±0.83	1.38*±0.16	0.44±0.28	45.50*±11.47	20.50±1.29	1330*±3.97	74.26*±5.83

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

Table 4. Erythrogram of Nile tilapia fish exposed to mercuric chloride $^1/_{10}$ LC_{50}/96-hr for 8 weeks

Time (weeks)	Group	RBCs (10^6/µl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
2	C	1.83±0.10	6.35±0.82	17.25±0.96	94.82±8.85	35.01±6.27	36.72±2.03
	T	1.38*±0.06	4.03*±0.29	14.18*±0.56	107.79±8.47	29.16±2.47	28.41±6.77
4	C	1.95±0.19	7.45±0.52	18.75±1.71	96.70±11.16	38.47±4.62	40.02±5.13
	T	1.30*±0.08	4.50*±0.42	13.78*±0.52	106.44±10.16	34.65±3.01	32.70±3.12
6	C	1.47±0.22	6.80±0.45	18.25±2.22	125.68±18.55	46.94±6.14	37.69±5.52
	T	1.04*±0.05	3.98*±0.42	15.70*±0.48	151.92*±8.23	38.63±8.59	25.26*±4.31
8	C	1.74±0.10	7.78±0.71	17.70±0.81	10.96±9.78	44.57±2.23	44.06±5.19
	T	1.01*±0.06	4.20*±0.52	13.25*±0.34	132.79*±6.44	41.88±6.52	31.45*±3.80

C = Control
T = Treated
* Significantly different from normal control, $p<0.05$

the experiment. Significant absolute lymphocytosis accompanied with normal values in monocytes, basophils and eosinophils were detected along the whole period of the experiment (Table 5). Serum urea and creatinine were increased significantly from the 2nd week until the end of the experiment. Value of total bilirubin showed significant increase in the 2nd and the 4th week of exposure. There significant elevation of both serums AST and ALT activates along the whole period of exposure in comparison to normal control .CK showed significant decrease in serum activity from the 2nd week of exposure to the 4th week. A highly significant increase than normal control was detected from the 6th week to the end of the experiment .A significant decrease in the glucose level in serum was detected from the 2nd week of exposure to the 6th week then significant hyperglycemia was detected in the 8th week (Table 6).

Table 5. Leukogram of Nile tilapia fish exposed to mercuric chloride $^{1}/_{10}$ LC_{50}/ 96hr for 8 weeks

Time (weeks)	Group	WBCs (10^3/µl)	Differential Leucocytic count				
			Heterophils (10^3/µl)	Lymphocytes (10^3/µl)	Monocytes (10^3/µl)	Eosinophils (10^3/µl)	Basophiles (10^3/µl)
2	C	9.50±1.29	1.80±0.93	7.60±0.91	0.11±0.21	0	0
	T	43.00*±4.16	5.19*±3.11	37.25*±3.13	0.54±0.13	0	0.05±0.15
4	C	9.50±1.29	1.84±0.56	7.56±0.37	0.11±0.22	0	0
	T	25.50*±1.91	3.07*±2.96	22.11*±2.94	0.30±0.17	0	0.01±0.05
6	C	9.75±0.96	1.91±0.59	7.72±0.57	0.12±0.12	0	0
	T	21.25*±2.75	1.97*±1.76	19.00*±1.81	0.29±0.06	0	0.01±0.1
8	C	9.00±1.41	1.78±0.35	7.12±0.49	0.10±0.24	0	0
	T	24.00*±6.73	3.09*±2.47	20.61*±2.48	0.28±0.14	0	0.02±0.1

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

Table 6. Some serum biochemical constituents of Nile tilapia fish exposed to $^{1}/_{10}$ LC_{50}/96 hr. of mercuric chloride for 8 weeks

Time (weeks)	Group	Urea (mg/dl)	Creatinine (mg/dl)	Total bilirubin (mg/dl)	AST (U/L)	ALT (U/L)	CK (U/L)	Glucose (mg/dl)
2	C	11.08±1.36	1.00±0.21	0.77±0.15	19.25±1.89	18.25±1.89	2991±7.85	84.75±7.38
	T	19.75*±2.09	1.90*±0.13	1.79*±0.10	37.50*±4.20	30.5*±9.15	1393*±8.14	62.71*±6.14
4	C	10.73±1.21	0.94±0.05	0.81±0.12	18.75±1.707	19.25±2.06	3000±7.08	90.96±4.31
	T	14.47*±1.53	1.72*±0.09	1.71*±0.44	45.50*±8.81	27.00*±4.00	2030*±4.70	67.38*±9.10
6	C	10.04±1.65	0.90±0.06	0.76±0.15	20.00±1.83	19.25±1.26	2998±6.83	88.83±2.87
	T	15.13*±2.28	1.80*±0.06	0.78±0.20	39.75*±5.50	28.50*±3.42	4231*±6.81	50.53*±9.86
8	C	9.27±0.85	0.87±0.06	0.79±0.14	19.25±1.26	18.75±1.71	3001±6.89	92.89±4.79
	T	17.40*±1.18	1.91*±0.22	1.17±0.42	40.25*±3.10	26.50*±3.79	4089*±6.12	131.45*±3.44

C = Control
T = Treated
* Significantly different from normal control, $p<0.05$

Short term exposure to sublethal dose $^1/_2$ LC_{50}/ 96 hr of copper sulfate in Nile tilapia

Red blood cells (RBCs) count, Hb content and PCV value revealed significant increase which appeared clearly in the 2nd and 5th day of exposure then decreased non significantly by the end of the experiment comparing to the normal control. Mean corpuscular volume (MCV) showed significant decline in the 2nd and 5th day of exposure to copper. Other red cell indices showed no changes. (Table7). Total leucocytic count showed significant decrease than normal control starting from the 5th day of exposure to the end of the experiment. Results of differential leucocytic count revealed non-significant absolute heterophelia along the whole period of exposure accompanied with significant absolute lymphopenia. There was non-significant decrease in the monocytic. No changes were detected in eosinophilic and basophilic counts along the whole period of exposure (Table 8).

Urea and creatinine results revealed significant elevation of urea and serum creatinine which appeared along the whole period of exposure. Total Bilirubin results revealed significant increase than normal control from the 2nd day to the 7th day of exposure. Serum AST and ALT results revealed significant elevation which appeared from the 2nd day to the end of the experiment. There was significant decrease in serum CK activity which started in the 2nd day of exposure to the 5th day then significantly increased at the end of the experiment .No change in the serum glucose

Table 7. Erythrogram of Nile tilapia fish exposed to copper sulfate ½ LC_{50}/ 96 hr. for 7 days

Time (days)	Group	RBCs (10^6/µl)	Hb (g/dl)	PC (%)	MCV (fl)	MCH (pg)	MCHC (%)
2	C	1.44±0.12	4.97±0.69	18.75±1.71	133.51±6.04	34.37±2.99	25.84±3.20
	T	1.82*±0.13	6.35*±0.75	21.75*±0.95	119.92*±6.08	34.89±2.54	29.16±2.65
5	C	1.3±0.10	5.15±0.75	17.25±1.89	135.71±6.64	39.58±6.78	29.08±3.95
	T	1.90*±0.14	6.30*±0.58	21.50*±0.76	113.47*±5.82	33.15±1.81	29.26±1.93
7	C	1.48±0.04	5.18±0.39	19.75±0.96	133.02±5.77	34.92±3.63	26.28±2.74
	T	1.46±0.04	5.13±0.22	18.67±0.39	127.95±3.37	35.09±0.91	27.45±1.29

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

Table 8. Leukogram of Nile tilapia fish exposed to copper sulfate ½ LC_{50}/ 96 hr. for 7 days

Time (days)	Group	WBcs (10^3/µl)	Differential Leucocytic count				
			Heterophils (10^3/µl)	Lymphocytes (10^3/µl)	Monocytes (10^3/µl)	Eosinophils (10^3/µl)	Basophiles (10^3/ µl)
2	C	18.75±0.96	3.57±0.24	14.99±0.76	0.19±0.021	0.00	0.00
	T	15.75±0.50	3.68±0.18	11.91*±0.36	0.16±0.01	0.00	0.00
5	C	18.25±0.50	3.46±0.15	14.95±0.52	0.20±0.03	0.00	0.00
	T	14.00*±0.81	3.55±0.13	10.27*±0.73	0.16±0.2	0.003±0.006	0.00
7	C	18.00±2.828	3.42±0.50	14.36±2.29	0.21±0.06	0.00	0.00
	T	13.25*±1.258	3.59±0.49	9.45*±0.84	0.18±0.04	0.003±0.006	0.00

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

level was detected in the 2nd day of exposure but significant hyperglycemia was detected from the 5th day of exposure to the end of the experiment (Table 9).

Long term exposure to sublethal dose (1/10 LC 50/96 hr.) of copper sulfate in Nile tilapia fish.

RBCs count, Hb content and PCV revealed significant increase than normal control on the 2nd, 4th and 8th week of exposure. No significant changes appeared on the 6th week. Values of MCV and MCH showed significant decrease in the 2nd, 6th and 8th week of exposure while no significant change was shown on the 6th week. MCHC showed no significant change along the whole period of exposure. (Table10) Total leucocytic count showed significant decrease which appeared from the 2nd, to the 8th week of the experiment. Values of differential leucocytic count revealed significant heteropenia in the 2nd and 8th week of exposure together with lymphopenia along the whole period of the experiment. Significant monocytosis appeared only on the 8th week of exposure. There were no detectable changes in basophils and eosinophils (Table11).

Urea and creatinine were increased significantly along the whole period of exposure .Total Bilirubin Results revealed no significant changes in the value of total bilirubin along the whole period of exposure .Results showed significant elevation of serum AST

Table 9. Serum biochemical constituents of Nile tilapia fish exposed to ½ LC_{50}/96 hr. of copper sulfate for 7 days

Time (days)	Group	Urea (mg/dl)	Creatinine (mg/dl)	Total billirubin (mg/dl)	AST (U/L)	ALT (U/L)	CPK (U/L)	Glucose (mg/dl)
2	C	8.58±0.63	0.85±0.08	0.57±0.25	18.00±2.94	15.75±1.50	2998±5.14	87.94±4.92
	T	14.01*±1.42	1.71*±0.21	0.87*±0.35	23.00*±3.26	34.50*±7.72	2660*±6.72	100.31±2.37
5	C	9.39±0.86	0.89±0.05	0.68±0.06	19.50±1.92	13.75±2.75	2989±5.27	87.82±2.01
	T	13.11*±1.94	1.63*±0.17	0.81*±0.34	33.25*±7.59	16.00*±4.69	2208*±4.82	188.60*±2.36
7	C	9.20±0.86	0.83±0.094	0.56±0.11	21.00±2.09	11.25±4.27	3008±6.04	97.10±2.96
	T	11.86*±1.134	1.47*±0.20	0.61*±0.29	32.75*±7.68	23.25*±6.65	5604*±3.88	328.29*±4.02

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

Table 10. Erythrogram of Nile tilapia fish exposed to copper sulfate $^{1}/_{10}$ LC_{50}/96 hr. for 8 weeks

Time (weeks)	Group	RBCs (10^6/µl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
2	C	1.57±0.05	4.98±0.35	21.25±0.50	135.00±4.45	31.57±1.65	23.43±1.90
	T	2.25*±0.13	6.00*±0.28	26.50*±1.914	117.73*±3.07	26.70*±1.70	22.69±1.09
4	C	1.42±0.04	5.25±0.34	23.50±1.29	164.58±5.52	36.82±2.92	22.42±2.36
	T	2.01*±0.14	6.22*±0.25	27.00*±0.81	134.21*±8.37	30.93*±1.76	23.08±1.36
6	C	1.51±0.12	5.05±0.57	22.00±0.82	145.74±6.39	33.41±3.25	23.01±3.04
	T	1.54±0.07	5.00±0.73	21.25±0.95	138.51±5.23	32.59±4.75	23.56±3.55
8	C	1.45±0.06	5.23±0.33	21.87±0.63	150.95±4.42	36.08±2.74	23.91±1.81
	T	2.09*±0.09	6.32*±0.55	24.00*±1.15	114.92*±8.65	30.20*±1.84	26.39±2.46

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

activity only on the 2nd week of exposure while no other changes were detected tell the end of the experiment. ALT activity showed no significant changes. There was significant decrease in serum CK activity from the 2nd week of exposure to the end of the experiment. Significant hyperglycemia was detected in the 4th week of exposure followed by significant hypoglycemia in the 6th and the 8th week (Table12).

DISCUSSION

Nile tilapia fish exposed to ½ LC_{50}/96hr. of mercuric chloride for one week showed significant decrease in RBCs count, PCV% and Hb concentration than normal control along the whole period of the experiment. These results agree with *Panigrahi and Misra (1978), Gill and Pant (1981), Mazhar et al. (1987) and Kumari and Banerjee (1993).*The observed anemia may be attributed to destruction of red

Table 11. Leukogram of Nile tilapia fish exposed to copper sulfate $^1/_{10}$ LC_{50}/96 hr. for 8 weeks

Time (weeks)	Group	WBCs (10^3/µl)	Differential Leucocytic count				
			Heterophils (10^3/µl)	Lymphocytes (10^3/µl)	Monocytes (10^3/µl)	Eosinophils (10^3/µl)	Basophils (10^3/µl)
2	C	19.00±2.58	3.75±0.58	15.06±2.00	0.19±0.04	0.00	0.00
	T	13.00*±1.32	2.99*±0.23	9.86*±1.09	0.15±0.02	0.00	0.00
4	C	21.00±1.83	4.10±0.28	16.67±1.51	0.23±0.06	0.00	0.00
	T	16.00*±1.36	3.74±0.34	12.05*±1.29	0.21±0.02	0.00	0.00
6	C	19.00±3.46	3.74±0.77	15.09±2.67	0.19±0.02	0.00	0.00
	T	14.25*±0.96	3.30±0.13	10.76*±0.80	0.18±0.05	0.00	0.00
8	C	18.00±3.65	3.53±0.82	14.30±2.83	0.17±0.02	0.00	0.00
	T	7.00*±3.46	1.56*±0.74	5.36*±2.68	0.08*±0.04	0.00	0.00

C = Control
T = Treated
*Significantly different from normal control, $p<0.05$

Table 12. Serum biochemical constituents of Nile tilapia fish exposed to $^1/_{10}$ LC_{50}/96 hr. of copper sulfate for 8 weeks

Time (weeks)	Group	Urea (mg /dl)	Creatinine (mg /dl)	Total bilirubin (mg/dl)	AST (U/L)	ALT (U/L)	CK(U/L)	Glucose (mg/dl)
2	C	9.27±0.89	1.20±0.083	0.85±0.13	13.25±2.5	8.00±3.27	2901±4.83	93.14±2.99
	T	13.85*±2.02	2.18*±0.06	1.61±0.59	17.75*±2.17	9.25±5.50	1195*±3.26	94.53±4.23
4	C	8.11±0.97	0.86±0.11	0.91±0.06	10.50±1.00	7.00±2.00	3105±4.05	88.77±5.65
	T	18.06*±4.87	2.49*±0.14	1.24±0.43	9.25±2.87	6.00±2.31	1247*±4.51	106.19*±2.28
6	C	8.92±1.42	0.84±0.17	0.92±0.09	12.25±2.87	6.50±1.29	3001±2.98	86.88±2.94
	T	12.27*±0.54	2.01*±0.037	0.96±0.22	10.0±2.45	5.50±1.91	1289*±3.27	76.56*±3.14
8	C	8.21±0.77	0.86±0.07	0.86±0.09	9.25±1.50	7.00±2.00	3127±3.92	93.32±2.96
	T	10.78*±1.47	1.93*±0.36	0.75±0.18	7.50±0.58	5.75±0.50	2105*±1.92	84.99*±1.50

T = Treated
C = Control
*Significantly different from normal control, $p<0.05$

cells and or defective utilization of iron.

Our study revealed decrease in the total leucocytic count due to significant heteropenia accompanied by significant absolute lymphopenia and monocytopenia. Our results agree with that obtained *by Storozhuk and Guleva (1983), Gill and Pant (1985).* Decrease of WBCs in acute toxicity due to heteropenia may be resulted from severe stress *(Larsson et al. 1980).* Lymphopenia and granulocytosis, have been detected after exposure to many pollutants *(Svobodova et al., 1996).*

Nile tilapia fish exposed to $1/_{10}$ LC_{50}/96hr. of mercuric chloride for 8 weeks showed significant decrease in RBCs count, PCV and Hb which agree with *Shakoori et al. (1994), Shah and Altindage (2004), Nilton et al. (2007) and Maheswaran et al. (2008).* This decrease may be attributed to decrease rate of production of blood cells or increased loss of these cells. *Gill and Epple (1993)* attributed anemia to (1) impaired erythropoiesis due to direct effect of metal on hematopoietic centers (kidney /spleen), (2) accelerated erythroclasia(erythrocyte fragmentation) due to altered membrane permeability and/or increased mechanical fragility and (3) defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions. Total leucocytic count showed significant increase appeared from the 2nd week to the end of the experiment. Values of differential leucocytic count revealed significant absolute heterophilia and lymphocytosis along the whole period of the experiment while there was no detectable change noticed in monocytes, basophils and eosinophils. These results coincide with those of *Joshi et al.(2002), Masud et al. (2005) and Oliveira et al. (2006).*The increase in WBCs observed in the chronic exposure attributed to increase number of lymphocytes could be a result of tissue damage caused by mercury or cytological shift resulting in increased large lymphocytes population *(Gill and Pant, 1985).*There are possibilities of direct action of Hg on the lymphocytes of Rosy barb fish (Barbus conchonius). The stimulation of human lymphocytes by ionic Hg leading to enhanced β–microglobulin production is already known *(Oshawa and Kimura, 1979)*Elevated serum urea and creatinine values in the acute and chronic toxicity experiments obtained results agreed with *Agrwal (1992), Berntssen et al. (2004)* and *Ayyat and El-Marakby(2005).* The content of serum urea is higher in captive fish than in wild fish. This indicates either an efficient metabolism of amino acids, or some disturbances from inefficient NH_3 removal in the cultured fish *(Svoboda, 2001)*. Elevation of serum urea and creatinine could be also attributed to necrosis in kidney and in the epithelial cells of collecting ducts. Increase urea could also be attributed to gill dysfunction *(Lockart and Metner, 1984)* since urea is excreted mainly through the gills *(Michael, 2002)*

Serum total bilirubin showed no changes in the acute toxicity while significant increase in the 2nd and 4th week of mercuric chloride chronic toxicity exposure was observed. *Badiei et al. (2010)* studied the effect of long-term low-dose administration of mercury (Hg) in sheep and found that the concentration of bilirubin (TBIL) was increased on day 70 when compared to the control group ($p<0.05$). Increase of bilirubin may be attributed to toxic effect of mercury that induced hepatocytes necrosis. Liver damage is indicated by elevated serum aspartate aminotransferase and alanine aminotransferase activity and may be associated with an increase in serum bile acid concentration *(West et al., 1987).* Hyperbilirubinaemia also may suggest obstruction of the bile ducts. In some forms of anemia, particularly of the hemolytic type, the level may rise due to the inability of the liver to pass the increased quantity of the pigment. The elevated levels of serum bilirubin in the present study suggest liver damage *(Jyothi and Narayan, 1999).*

Results of acute mercury toxicity revealed significant elevation of serum AST started from the 5th day of exposure to the end of the experiment while serum ALT was elevated only in the 2nd day of ex-

posure and returned to normal by the end of the experiment. Chronic mercury toxicity results revealed significant elevation of both serums AST and ALT along the whole period of toxicity. This result agree with that obtained by *Helmy et al. (1980a), Suresh et al. (1991) and Muazzez et al. (2008).*The significant changes in activities of these enzymes in blood plasma indicate tissue impairment caused by stress *(James et al., 1991 and Svoboda, 2001).*

Serum CK activity revealed significant decrease started from the beginning of exposure until the end of the experiment in the acute toxicity. In chronic toxicity experiment, significant decrease in serum CK was observed from the 2nd week of exposure to the 4th week then a high significant increase than normal control was detected from the 6th week till the end of the experiment. Increase of CK agree with the result obtained by *Ali (2004)* and could be attributed to damage of the muscles.

Glucose value in acute toxicity with mercury showed significant decrease than normal especially from 5th day to the end of the experiment. Same result was obtained in the chronic toxicity except at 8th week it started to be significantly higher than normal control .This decrease of glucose level agree with the result obtained by *Sastry and Rao (1981)* while the increase in last week agree with the result obtained by *Bleau et al. (1996).*The increase of glucose might resulted from an increase in plasma catecholamines and corticosteroid hormones *(Pickering, 1981).*The pronounced muscular exertion due to the stress exerted by toxicant resulted in the breakdown of tissue glycogen necessary to meet the energy demand, probably through the process of glycogenolysis causing hyperglycemia in fish. Hyperglycemia and reduction in the glycogen of liver and muscle of fish after exposure of various pollutants were also registered *by Nakano and Tomlinson (1967),*

Hypoglycemia most probably could be attributed to renal failure to reabsorb 100% glucose and its redistribution to the tissue cells via the blood capillaries. The renal impairment could be due the toxic effects of heavy metals present in the ambient habitat. There are many such reports about renal tubules impairment as a result of metal intoxication. Renal dysfunction due to heavy metal intoxication has been reported in children *(Friberg et al., 1979).*

Hematological picture of Nile tilapia exposed to acute toxicity of copper sulfate revealed significant increase in RBCs count, Hb and PCV which appeared clearly in the 2nd and 5th day of exposure. This comes in agreement with *Williams and Wooten (1981), Dick and Dixon (1985), Singh and Reddy (1990),* Also in chronic toxicity of copper there was significant increase in RBCs count, PCV and Hb especially in the 2nd, 4th and 8th week. This comes in agreement with *Joachim et al. (2007).* Such increases may be attributed to stress reaction that cause an osmotic misbalance and changes in the regulatory system of ionic interchange which can diminish pH of blood and increase the volume of erythrocytes and subsequently the percent of haematocrit.

Total leucocytic count of Nile tilapia fish in acute copper sulfate toxicity showed non-significant leucopenia starting from the 2nd day of exposure to the end of the experiment. This resulted from non-significant lymphopenia and heteropenia which agree with the results obtained by *Khangarot and Tripathi (1991),* In the chronic toxicity experiment, result of total leucocytic count showed significant decrease which appeared from the 2nd week to the end of the experiment. Changes were attributed to significant lymphocytopenia and significant heteropenia especially in the 2nd and 8th week with non-significant decrease in monocytes. Our result comes in agreement with *Dick and Dixon (1985), Dethloff et al. (1999)* It is known that cortisol secreted during stress reaction shortens the life span of lymphocytes, promotes their apoptosis *(Wyets and Verburg, 1998)* and reduce their proliferation *(Espelid et al. 1996).* Leukopenia is also a common reaction of fish to metal exposure.

The determined levels of serum urea and creatinine in Nile tilapia exposed to acute copper toxicity revealed significant elevation from the 2nd to the 7th day of exposure. Similar results were obtained in the chronic copper toxicity. Urea increase agrees with that obtained by *Muazzez et al. (2008)*. Blood urea nitrogen is a major nitrogen-containing metabolic product of protein catabolism and acts as a major osmolyte, therefore it can be used as a sensitive tool to predict the gill and kidney dysfunction in fish.. Elevation of serum urea and creatinine could be attributed to the damage in kidney and in the epithelial cells of collecting ducts. Also increase urea could be attributed to gill dysfunction *(Lockart and Metner, 1984)*.

Result of total bilirubin in acute copper sulfate toxicity revealed significant increase than normal control along the whole period of exposure while in chronic toxicity revealed no significant change. This elevation agrees with that obtained by *Badiei et al. (2010)*. Increase of bilirubin may be attributed to damage of hepatocytes which reduce hepatic function and may be associated with an increase in serum bile acid and bilirubin concentration *(West et al., 1987)*.

Results of acute copper toxicity revealed elevation of serum AST and ALT from the 2nd day to the end of the experiment. This agree with results obtained by *Nemcsók and Hughes (1988),* Folmar (1993), *Muazzez et al. (2008)*and *Oner et al. (2008)*. Chronic copper toxicity results revealed significant elevation of serum AST only on the 2nd week of exposure while no other changes were detected to the end of the experiment. Also ALT results showed no significant changes. The results agreed more or less with that obtained by *Karan et al. (1998) and Zsolt et al. (2001*). Increase in AST activity in both acute and 2nd week of chronic toxicity could be attributed to the tissue impairment caused by stress (*James et al., 1991 and Svoboda, 2001)* or could be as a result of hepatocellular damage or cellular degradation by the heavy metal to liver, heart or muscle *(Yamawaki et al., 1986)*.

Result of CK in acute copper toxicity revealed significant decrease in the 2nd and 5th day followed by significant increase in the 7th day while chronic toxicity revealed significant decrease along the whole experiment. The decrease in CK activity agree with *Ching-Feng et al. (2002) and Almeida et al. (2001)* while the increase in CK agree with *Adham (2002)* who reported increase in the Ck in metal pollution in the Nile and Delta lakes in *Clarias lazera*. Weakened efflux of the muscle enzyme may cause subnormal CK level in the serum and may be resulted also from reduced physical activity due to illness or reduced muscle mass accompanying with wasting muscle diseases (*Sidney, 1998)*.

Glucose level showed significant increase in copper acute toxicity especially from the 5th day of exposure, while in the chronic toxicity it showed increase in the 2nd week and became significant in the 4th week followed by significant decrease in the 6th to 8th week of exposure. Increase in glucose level agree with Dethloff *et al. (1999)*, *Monteiro et al. (2005),* The decrease in the glucose level agree with *Dhanapakiam and Ramasamy (2001)*. The rapid rise in plasma glucose concentration following an acute stressor has been associated with the activation of the Hypothalamus-Sympathetic-Chromaffin cell (HSC) axis (*McDonald and Milligan, 1997)*. The response to stress in teleost fish is similar to that of the terrestrial vertebrates.

Meanwhile, a decrease of glucose indicates the exhaustion of energy (glycogen) resources and subsequently, the worsening of an organism status. Prolonged exposure to stressors in high concentration makes it difficult for a fish to adapt to and creates weakness characterized by decrease in serum cortisol and glucose levels.

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