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PROOF

Alterations of Growth Performance and Blood Chemistry in Nile Tilapia (*Oreochromis Nuoticus*) Affected by Copper Sulfate in Long-Term Exposure

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Abstract

The objective of this study was to evaluate the effects of copper sulfate ($CuSO_4$.(5 H_2O)) on the Nile tilapia (Oreochromis niloticus). Median (LC_{50}) and minimum (LC_{1O}) lethal concentrations of copper sulfate on Nile tilapia were determined via acute tests. LC_{50} values of copper sulfate for the 48th, 72nd and 96th hours were 13.15, 12.95 and 12.85 mg/L, respectively. LC_{10} values of 12, 11 and 9.5 mg/L were obtained for similar exposure periods (48th, 72nd and 96th hours), respectively. The effects of 1.5 mg/L dose of copper sulfate on growth rate, gross clinical observations and blood biochemical parameters of Nile tilapia were evaluated after 35, 65 and 95 days of exposure periods. The differences of growth rates between control and copper sulfate-exposed groups were not significant (P>0.05) at the end of 35, 65 and 95 days. Continuous exposure of Nile tilapia to 1.5 mg/L concentration of copper sulfate in water solution for three periods (35, 65 and 95 days) decreased the total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (except the elevation in the exposed group at the end of 95 days period), magnesium (Mg^{+2}), calcium (Ca^{+2}), iron (Fe^{+2}), potassium (K^{+}), sodium (Na^{+1}), chloride (Cl^{-}) and phosphate values in serum. The cholesterol, low density lipid (LDL), triglyceride, globulin, creatinine, alkaline phosphatase (ALP), total bilirubin, direct bilirubin, uric acid and blood urea nitrogen (BUN) concentrations in the serum increased in copper sulfate-exposed fish.

Keywords: Nile tilapia, copper sulfate, LC_{50}, LC_{10}, blood, metabolite, electrolyte, growth.

Uzun Süre Bakır Sülfata Maruz Kalan Nil Tilapia (*Oreochromis Nuoticus*) Balığının Büyüme Performansı ve Kan Kimyasındaki Değişimler

Özet

Bu çalışmamızın amacı bakır sülfatın ($CuSO_4$.($5H_2O$)) Nile Tilapia (Oreochromis niloticus) üzerindeki etkilerini belirlemektir. Bakır sülfatın Nile tilapia üzerindeki medyan (LC_{50}) ve minimum (LC_{1O}) ölümcül konsantrasyonları akut testlerle belirlenmiştir. Bakır sülfatın 48, 72 ve 96 saatlerdeki LC_{50} değerleri sırası ile 13.15, 12.95 ve 12.85 mg/L idi. Aynı sürelerde elde edilen LC_{10} değerleri de sırası ile 12, 11 ve 9.5 mg/L idi. 1.5 mg/L bakır sülfat dozunun Nile tilapia'da büyüme, bütüncül klinik görünüm ve kan biyokimyası parametreleri üzerindeki etkileri 35, 65 ve 95 günlük maruziyetler üzerinden değerlendirilmiştir. Kontrol ve bakır sülfat uygulama grupları arasında büyüme oranları arasındaki fark 35, 65 ve 95 gün sonunda anlamlı değildi (P>0.05). 1,5 mg/l'lik bakır sülfat konsantrasyonuna sürekli maruziyet Nile tilapia'da 35, 65 ve 95 günlük süreler sonunda toplam protein, albümin, aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), glikoz (95 günlük gruptaki artış dışında), magnezyum (Mg^{+2}), kalsiyum (Ca^{+2}), demir (Fe^{+2}), potasyum (K^{+}), sodyum (Na^{+1}), klor (Cl^{-}) ve fosfatın serumdaki değerlerini düşürmüştür. Kolesterol, düşük yoğunluklu lipit (LDL), trigliserit, globülin, kreatinin, alkalin fosfataz (ALP), total bilirubin, direkt bilirubin, ürik asit ve üre azotu (BUN) konsantrasyonları bakır sülfata maruz kalan balıkların serumlarında yükselmiştir.

Anahtar Kelimeler: Nile tilapia, bakır sülfat, LC_{50}, LC_{10}, kan, metabolit, elektrolit, büyüme.

Introduction

Copper sulfate is an algicide, and used in order to treat parasitic and fungal diseases in fish. Copper is present in most of aquatic environments; however, it is also toxic for fish and its toxic effects have been studied on all fish species. In recent years, the impacts of industrial effluents on aquatic ecosystems have become much severe due to the development of industries. Industries such as copper, aluminum and iron-steel factories release copper and its compounds into water, and it may cause serious problems. Thus,

the control of toxic substances in environment is now an urgent issue. One of the pollutants leading to heavy metal pollution is the copper element. It has different effects on different species (Mazon *et al*., 2002). In studies on exposure of fish to copper, it has been shown that this exposure leads to negative effects on body weight (Hamilton *et al*., 1977; Schjolden *et al*., 2007), and on digestive enzymes and lipase (Sastry and Gupta, 1978).

Tilapia species are important fish reared in the Mediterranean region in southern Turkey. These fish are economically important, and a research on their physiological response to toxicants should be conducted. In addition, tilapia species have certain physiological capabilities, so they can easily adapt to different environmental conditions. In this study, we used the blood parameters of the fish as indicators of their physiological state. Fish blood parameters have been increasingly used in environmental monitoring softwares as valuable indicators of physiological changes in the presence of toxicants (Mazon *et al*,. 2002; Schjolden *et al*., 2007).

The aims of the present study were to test acute toxicity of copper sulfate in the Nile tilapia (*Oreochromis niloticus*), to observe the effects of copper sulfate on the growth rate of the fish and to investigate the gross and chemical clinical effects of exposure to copper sulfate on the Nile tilapia.

Materials and Methods

The copper sulfate ($CuSO_4.(5\ H_2O)$) was provided by Sigma®Aldrich Biotechnology LP and Sigma-Aldrich Co, Germany.

Laboratory static renewal tests were conducted in order to determine the median (LC_{50}) and the minimum (LC_{10}) lethal concentrations of copper sulfate in the Nile tilapia (*Oreochromis niloticus*).

Ten fish of similar sizes were sampled randomly, and placed in aquaria. After 15 days of acclimatization, the fish were exposed to various copper sulfate concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 mg/L) for 48, 72 and 96 h. The control and each of treated groups were run in triplicate, and a total of 450 Nile tilapia fish were placed 10x10 in each aquarium. During the experiment, dead fish were removed, and mortality was recorded after 48, 72 and 96 h. The LC_{50} values of copper sulfate for Nile tilapia were calculated by using the Trimmed Spearman-Karber method applied by Hamilton et al. (Statistically Analytic System, 1993). The LC_{10} values were determined by utilizing linear regression on the percentage of the Nile tilapia mortality data.

The total of 240 Nile tilapia fish was acclimatized to laboratory conditions for 15 days. The fish having average body weight of 46.10 ±2.1 g were then divided into the experimental and control groups. They were exposed to 1.5 mg/L of copper sulfate in water solution. In the first experiment of the 35 days period, a total of 40 fish were placed in four aquaria for copper sulfate-exposed treatment. The same number of placement was applied to the control group as in the experimental group. The same procedures were applied to both of the remaining 65 and 95 days period experiment groups and the control groups. During the experiment, all fish were fed regularly with extruded carp pellets. After each 35, 65 and 95 days of trial periods, fish were weighed, and the average growth rate of each fish group was calculated. At the end of each period, necropsy was performed on 10 fish from each treatment, and gross clinical signs were recorded.

This study has been carried out in newly established Toxicology Institute of Hafik Vocational School of Cumhuriyet University. The experiments have been performed within 100 L glass-aquariums (85x40x30 cm). 10 fish have been placed in each of aquariums. Each of work has been carried out with 4 repeats. Before starting the experiment, the fish have been acclimatized to laboratory conditions for 15 days. It has been planned for 3 different durations as 35, 65, and 95 days. During the trial period, water temperature was 26°C with pH 8.32, 6.18 mg/L dissolved oxygen content, 255 mg/L calcium carbonate ($CaCO_3$ alkalinity and 33 total hardness (Fr.). The water in aquaria has been renewed every 2 days and the Cu concentration has kept constant by adding copper sulfate. Approximately 2 mL blood sample was drawn from the caudal vein by puncture, and each sample was immediately transferred into individual silicone coated Vacutainer Tubes (Becton Dickinson and Company, Belgium; not containing EDTA). Blood samples were centrifuged promptly at 3,100g- for 10 min, and the serum was removed with a disposable transfer pipette. Concentrations of magnesium (Mg^{+2}), chloride (CI), potassium (K^+), sodium (Na^+), phosphate, calcium (Ca^{+2}), iron (Fe), cholesterol (CHOL), triglyceride (TG), low density lipid (LDL), albumin (ALB), globulin (GLO), creatinine (CRE), total bilirubin (TBIL), direct bilirubin (DBIL), total protein (TP), uric acid (UA), blood urea nitrogen (BUN), glucose (GLC), alkaline phosphatase (ALP), aspartate aminotransferase (glutamate oxalacetate transaminase, AST-GOT) and alanine aminotransferase (glutamate pyruvate transaminase, GPT-ALT) were determined by using an auto-analyzer (Merck-Mega/Toshiba, Japan).

The differences among the growth rates of the fish groups were tested with the variance analyses, and the averages of the groups were analyzed using Duncan's test (Cnaani *et al*., 2000). The data obtained from blood analyses were subjected to non-parametric analysis of variance by using the Minitab User Guide software (Cnaani *et al*., 2000). A value of $P<0.05$ was considered significant.

Results

According to the static renewal method for the

acute toxicity testing, the LC_{50} values of copper sulfate for the Nile tilapia were obtained. The LC_{50} values for the 48, 72 and 96 h were 13.15, 12.95 and 12.85 mg/L, respectively. The LC_{10} values for the same time periods were 12, 11 and 9.5 mg/L, respectively.

In 35-day group, the fish group exposed to copper sulfate tolerated the chemical compound without mortality. Gross external or internal sign in the exposed fish group was not observed during necropsy. However, there were necrotic spots in the yellowish liver of the Nile tilapia exposed to copper sulfate at the end of 65 days. A light red in the spleen, pale gills and liver were observed in the fish exposed to copper sulfate after 95 days period.

After 35, 65 and 95 days periods, the growth rate differences were not statistically significant between the control and the copper sulfate-exposed treatments (Table 1).

Despite the fact that the mean triglyceride level was lower in 65-day copper-sulfate group, the serum cholesterol, LDL and triglyceride concentrations in the copper-sulfate group were significantly higher than those of control group during all three periods (Table 2). The serum globulin, total bilirubin, direct bilirubin, creatinine, uric acid and BUN levels in the exposed group, except a decrease in total bilirubin in 95 days period, were significantly higher than those in the control group in each period (Table 2). The total protein and albumin concentrations in the serum of the copper sulfate-exposed fish were significantly less than those of the control fish after three periods

Table 1. The end of 35,65 and 95 days, average growths of Nile tilapia groups (n= 40; mean ± SD; *P < 0.05)

Group	Growth rate (%)		
	In 35 days	In 65 days	In 95 days
Exposed to copper sulphate	14.3±3.0	24.92±8.4	27.60±5.4
Control	16.3±1.4	27.28±2.9	30.42±5.2

Table 2. Non-parametric analysis of variance of serum metabolites offish groups (n = 30, mean ± SD). Superscripts in a row with different letters represent significant differences (P <0.05)

Test	Periods (day)	Group	
		$CuSO_4(5H_2O)$	Control
ALB (mg/dL)	35 d	1.18^{b}± 0.007	1.37^{b}± 0.008
	65 d	1.06^{a}± 0.009	1.35^{b}± 0.009
	95 d	0.78^{a}± 0.007	1.32^{b}± 0.007
GLO (mg/dL)	35 d	2.43^{b}± 0.004	1.68^{a}± 0.001
	65 d	2.60^{b}± 0.003	1.68^{a}± 0.001
	95 d	2.67^{b}± 0.003	1.68^{a}± 0.005
CRE (mg/dL)	35 d	0.34^{b} ± 0.001	0.22^{a}± 0.001
	65 d	0.38^{b}± 0.001	0.20^{a}± 0.001
	95 d	0.41^{b}± 0.001	0.21^{a}± 0.001
TBIL (mg/dL)	35 d	0.73^{b}± 0.008	0.41^{a}± 0.010
	65 d	0.74^{b}± 0.054	0.43^{a}± 0.008
	95 d	0.12^{a}± 0.001	0.43^{b}± 0.008
DBIL (mg/dL)	35 d	0.47^{b}± 0.009	0.21^{a}± 0.006
	65 d	0.52^{b}± 0.007	0.20^{a}± 0.007
	95 d	0.57^{b}± 0.008	0.20^{a}.4:0.003
TP (mg/dL)	35 d	3.54^{a}±0.014	4.21^{b}±0.011
	65 d	3.26^{a}± 0.009	4.15^{b}± 0.009
	95 d	3.10^{a}±0.012	4.13^{b}± 0.009
CHOL (mg/dL)	35 d	135.20^{b}±0.33	95.50^{a}±0.20
	65 d	123.40^{b}±0.23	100.19^{a}±0.13
	95 d	112.80^{b} ±0.41	99.57^{a}i0.20
TG (mg/dL)	35 d	66.60^{b}±0.29	62.40^{a}±0.28
	65 d	57.31^{a}±0.18	59.97^{b}±0.14
	95 d	7140^{b}±0.20	60.02^{a}±0.14
LDL (mg/dL)	35 d	86.501^{b}±0.89	77.950^{a}± 0.77
	65 d	110.602^{b}±0.41	79.609^{a}± 1.50
	95 d	136.600^{b}±0.42	80.010^{a}±0.27
UA (mg/dL)	35 d	3.89^{b}± 0.006	1.27^{a}± 0.001
	65 d	3.85^{b}± 0.002	1.36^{a}± 0.001
	95 d	3.94^{b}± 0.001	1.36^{a}± 0.002
BUN (mg/dL)	35 d	2.30^{b}± 0.009	2.12^{a}± 0.007
	65 d	2.60^{b}± 0.001	2.10^{a}± 0.001
	95 d	2.80^{b}±0.001	2.10^{a}± 0.001
GLC (mg/dL)	35 d	121.80^{a}±0.34	135.80^{b}±0.29
	65 d	135.60^{a}±0.20	137.40^{b}±0.20
	95 d	$148.30^{b,}$±0.25	136.90^{a}±0.15

(Table 2). At the end of both 35 and 65 days periods, the mean blood glucose levels in both tilapia groups exposed with copper sulfate were significantly less than those in the control Nile tilapia (Table 2). In contrast, the mean glucose concentration in the blood of the exposed group significantly increased after 95 days period (Table 2).

The serum ALP activity of the exposed fish group was significantly higher than that in the control fish after each period (Table 3). The serum AST and ALT activities and magnesium, potassium, sodium, chloride, iron, calcium and phosphate concentrations were significantly lower in the fish group exposed to copper sulfate compared with those of the control group at the end of three periods (Table 3).

Discussion

The LC_{50} values of the Nile tilapia suggested that it was one of the most toxicant tolerant freshwater species. This result agrees with the data described in (Qureshi and Saksena, 1980). As stated in (Nussey *et al*., 1995; Bashir *et al*., 2013; Sağlam *et al*., 2014), the copper has significant toxic effects on fish even at low concentrations. In addition, the partial LC_{10} values of copper sulfate were higher when compared with those of LC_{50} values in this study. Our results appeared at 26°C water temperature, with pH 8.32, 6.18 mg/L dissolved oxygen content, 255 mg/L alkalinity (at high alkalinity) and 33 total hardness (Fi\). The 96-h LC_{50} values of copper sulfate to Tilapia mossambica have been reported to be 1.5 mg/L in water having pH 8.5, 25°C temperature, 115 mg/L hardness and 98 mg/L alkalinity (Mukhopadhyay and Konar, 1984); 18 mg/L in water including pH 8.5, 27°C temperature, 268 mg/L hardness and 115 mg/L alkalinity (Strauss, 2003), The 96-h LC_{50} values of the blue tilapia exposed to copper sulfate were reported to be 43.1 mg/L in water with pH 8.7, 20.1°C temperature, 114 mg/L hardness and 224.9 mg/L alkalinity; 6.6 mg/L in water with pH 8.4, 20.1°C temperature, 57.5 mg/L hardness and 111.8 mg/L alkalinity; and 0.7 mg/L in water with pH 8.1, 20.1°C temperature, 28.2 mg/L hardness and 57.1 mg/L alkalinity (Perschbacher and

Table 3. Non-parametric analysis of variance of serum enzymes and electrolytes offish groups

Test	Periods (day)	Group	
		$CuSO_4(5H_2O)$	Control
ALP (U/dL)	35 d	465.20^{b}±0.58	387.20^{a}±0.13
	65 d	483.90^{b}±0.27	393.00^{a}±0.15
	95 d	497.10^{b}±0.26	394.00^{a}±0.17
AST (U/dL)	35 d	128.30^{a}±0.04	210.40^{b}±0.03
	65 d	121.40^{a} ±0.02	205.60^{b}±0.02
	95 d	115.00^{a}±0.03	206.20^{b}±0.02
ALT (U/dL)	35 d	20.22^{a}±0.03	41.12^{b}±0.04
	65 d	19.35^{a}±0.02	37.08^{b}±0.03
	95 d	20.01^{a}±0.05	38.81^{b}±0.03
Mg^{2+} (mg/dL)	35 d	4.52^{a}± 0.005	6.65^{b}± 0.001
	65 d	4.68^{a}± 0.004	6.59^{b}± 0.002
	95 d	4.61^{a}± 0.002	6.61^{b}± 0.002
CI(mmol/dL)	35 d	119.60^{a}±0.52	156.80^{b}±0.13
	65 d	126.40^{a}±0.32	150.50^{b}±0.21
	95 d	116.00^{a}±0.16	150.40^{b}±0.22
K^{+} (mg/dL)	35 d	4.45^{a}± 0.011	7.03^{b}± 0.009
	65 d	4.96^{a}± 0.021	7.08^{b}± 0.007
	95 d	4.29^{a}± 0.015	7.05^{b}± 0.009
Na^{+} (mg/dL)	35 d	141.01^{a}±0.35	210.92^{b}±0.14
	65 d	154.30^{a}±0.25	202.20^{b}±0.23
	95 d	148.00^{a}±0.15	$20l.90^{b}$±0.21
Phosphate (mg/dL)	35 d	7.27^{a}±0.08	11.17^{b}±0.07
	65 d	7.83^{a}±0.07	11.77^{b} ±0.08
	95 d	6.30^{a}±0.09	11.52^{b}±0.09
Ca^{2+} (mg/dL)	35 d	7.64^{a}± 0.009	8.22^{b}± 0.007
	65 d	8.02^{a}± 0.007	8.75^{b}± 0.009
	95 d	7.85^{a}± 0.009	8.69^{b}± 0.008
Fe (mg/dL)	35 d	18.13^{a}±0.09	29.53^{b}±0.12
	65 d	13.23^{a}±0.18	32.51^{b}±0.20
	95 d	10.02^{a} ±0.15	32.93^{b}±0.15

(n = 30, mean ± SD). Superscripts in a row with different letters represent significant differences (P<0.05).

Wurts, 1999). High calcium hardness of the water can minimize the toxic effects of copper on fish (Sciera *et al*., 2004; Carvalho and Fernandes, 2006). When data from other studies were compared with our data, it was demonstrated that the typical acute toxicity response of copper sulfate, in which toxicity to tilapia could be high as pH, total alkalinity and total hardness decrease. However, these criteria may vary between fish species and life stage, and the toxicity of copper to Prochilodus scrofa in water with pH 8.0 was observed higher than that in pH 4.5 by Carvalho and Fernandes (Carvalho and Fernandes, 2006). Perschbacher (Perschbacher, 2005) observed an inverse relationship between the toxicity of copper sulfate and water temperature for channel catfish. Temperature, pH and hardness are important variables influencing copper toxicity, and should be considered before treatment.

In this study, copper sulfate caused an insignificant decrease in the growth rate of the Nile tilapia, which is consistent with other studies (Rabago-Castro *et al*., 2006; Wu *et al*., 2007). Our results showed that 1.5 mg copper sulfate/L may safely be exposed to the Nile tilapia for 35 days without any significant effect on the growth of the fish, and the decreases of body weight gain were also insignificant at the end of 65 and 95 days periods. Ali *et al*. (2003) reported that significant decreases were observed in the total weight gain and specific growth rate of the O. niloticus reared in water containing different concentrations of copper; thus, these decreases were linearly correlated with the increase of the copper level in water.

The serum LDL contents in the fish exposed to copper sulfate increased by 11% after 35 days and 38.9% after 65 days and 70.7% after 95 days. The triglyceride value in the serum of the exposed fish group increased 6.7% in 35 days and 16% after 95 days, although a decrease of triglyceride concentration was observed 4.4% in the exposed group after 65 days. The elevations of the serum cholesterol concentrations in the exposed group were 41.6% after 35 days and 23.2% after 65 days; however, there was a 13.3% decrease in the exposed group at the end of 95 days. Our results were parallel to the findings reported in copper sulfate exposure (Chen *et al*., 2004), nephritic syndrome and glycogen storage disease characterized by liver dysfunction (Chen *et al*., 2002; Yang and Chen, 2003), toxicity of heavy metals (Adham *et al*., 2002; Yang and Chen, 2003) and pesticide studies (John, 2007). The gross clinical signs in the liver of the exposed fish with the changes, which was observed as 4% in triglyceride, 5% in cholesterol and 26% in LDL, were detected after 65 and 95 days; however, the changes in the serum lipids, which increased up to 41.6% of cholesterol and 62% of LDL and which decreased down to 20% of triglyceride, did not affect the health of the Nile tilapia during 35 days period. It is known that cholesterol, lipoproteins and triglyceride values are linked with the metabolism of lipids and functions of the liver and kidney (Yang and Chen, 2003). The increases of cholesterol content and the decreases in soluble protein and glycogen levels suggested the possible involvement of carbon skeleton of amino acids and acetyl CoA into cholesterol biosynthesis (Jain, 1999; Aride *et al*., 2007), which could have provided body weight gain and normal carbohydrate metabolism.

The low concentrations of the total protein in the serum of the fish exposed to copper sulfate, which were a 63.4% decrease in 35 days, a 69.6% decrease after 65 days period and a 73.4% decrease at the end of 95 days period, might have resulted from the exposure of copper sulfate. Total protein is used in order to evaluate protein metabolism; low concentrations may occur with nephritic syndrome (Chen *et al*, 2002) and liver disorder (John, 2007).

The changes of the serum albumin in the exposed fish were a 13.9% drop after 35 days period, a 21.5% decrease during 65 days period and a 40.9% decrease in 95 days. The percentages of the changes in the serum albumin concentrations of the fish group exposed to copper sulfate showed an increasing trend during periods from 35 to 65 and 95 days. Our results show that the decreases of albumin concentrations and the elevations of cholesterol and LDL in the serum of the exposed fish are present as was previously reported (De Smet *et al*., 1998). Measurement of serum albumin is of considerable diagnostic value in laboratory fish as it relates to general nutritional status, the integrity of the vascular system and liver function (Gopal *et al*., 1997). Hypoalbuminaemia in fish may be observed in the exposure of pollutants and other stress situations (Gopal *et al*., 1997; Chen *et al*., 2004).

The serum globulin concentrations in the exposed fish groups increased by 104.2% at the end of 35 days, 114.3% after 65 days period and 118.5% in 95 days period. The toxicity of heavy metals could cause an increase in the serum globulin level of fish. Exposure to heavy metals had a detrimental effect on the immunological response in fish (Gopal *et al*., 1997). The increases in serum globulin levels of 35-, 65- and 95-day groups in proportion to control group have shown similarities with findings of Gopal *et al*. (1997) The serum total bilirubin values increased by 78% during 35-day period and 72.1% during 65-day period; however, there was a 72.1% decrease in the total bilirubin concentration of the serum in the exposed group during 95-day period. The direct bilirubin levels in the serum of the fish exposed to copper sulfate elevated 123.8% in 35 days, 160% after 65 days period and 185% at the end of 95 days. Significant increases of the serum globulin, total bilirubin (except the decrease in total bilirubin of the 95-day exposure group) and direct bilirubin concentrations in the exposed fish groups may be the result of the effects of copper sulfate. Direct bilirubin is an amount of the soluble bilirubin in water. On the

contrary, total bilirubin comes to the muscle and the liver by binding to the albumin and globulin in the serum through filtration by the kidney. In addition, bilirubin is a predominant bile pigment found in the circulation in the Nile tilapia derived from disruption of hemoglobin (Folmar, 1993). Serum bilirubin levels of fish could change with hepatocellular disease (Chen *et al*., 2004) and the effect of mycotoxin (Van Vuren *et al*., 1994).

At the end of 35 days, the elevations of the serum creatinine, uric acid and BUN value concentrations in the exposed fish were 54.5%, 206.3% and 8.5%, respectively. After 65 days, the creatinine, uric acid and BUN amounts in the serum of the exposed fish increased 90%, 183.1% and 23.8%, respectively. In 95 days, the increases of creatinine (95.2%), uric acid (189.7%) and BUN (33.3%) were present in the serum of the fish exposed to copper sulfate. The high creatinine, BUN and uric acid might be expected in the serum of the exposed fish, as has been reported with exposures of copper (Chen et al., 2004), pesticides (Jain, 1999), other heavy metals and nitrate (Adham *et al*., 2002). The increases in serum BUN and creatinine concentrations have frequently been used in fish as an indicator of gill and kidney dysfunction (Adham *et al*., 2002; Yang and Chen, 2003), because there is a relationship between exposure to heavy metals and kidney disease. The serum uric acid, BUN and creatinine significantly increased in the copper sulfate-exposed fish in our study. This refers to a kidney failure and increased muscular tissue catabolism. The present data suggest that copper-exposed fish had glomerular dysfunction rather than tubular insufficiency. This finding is in parallel with the fact that the most prominent rise among serum nitrogenous compounds in these fish was reported for uric acid. It could be suggested that the branchial excretion of uric acid and other nitrogenous compounds was inhibited leading to an accumulation of uric acid in blood. This leads to confusion about the role of uric acid and BUN levels in assessing fish health. Any gross abnormality was not in the Nile tilapia although significant changes were caused in serum proteins, bilirubins and nitrogenous compounds following the exposure to copper sulfate after 35 days. However, clinical signs in gills, liver and kidney were present with the changes of these biood parameters in the samples of the exposed fish group after both long terms of 65 and 95 days.

The low serum glucose levels in tilapia group exposed to copper sulfate, which decreased by 10.3% after 35 days, might have resulted from hypoglycemia due to the increase or decrease in the activities of liver enzymes under stress caused by copper sulfate. These results were similar to those reported in long-term copper exposure in fish (Folmar, 1993). The increases of glucose concentrations were observed as 8.3% at the end of 95 days. In the present study, the elevated serum glucose concentrations might have resulted from hyperglycemia induced by long-term exposure to copper sulfate in the exposed fish. These theories are corroborated by the results of serum enzyme analyses showing the significant increases in ALP levels of the serum of the exposed fish groups, and the significant decreases in AST and ALT levels of the exposed groups. In addition, the decreases in blood glucose may account for the degeneration of muscular tissue. Blood glucose level in fish is known to be very useful as a criterion for diagnosis of the function of liver and muscle tissues (Folmar, 1993; Yang and Chen, 2003; Chen *et al*., 2004), and it could increase in fish exposed to copper (Pepelinjak *et al*., 2002; Nemcsok and Hughes, 1988). The decreased serum AST and ALT values and increased serum ALP activity of fish might be observed in copper exposure (Chen *et al*., 2004) as in our study. When damage occurs in tissues due to the heavy metal, ALT and AST aminotransferase enzymes, which are normally within the cell, have leaked out of the cell in order to neutralize the toxic matter. The serum ALT concentrations in the Nile tilapia group exposed to copper sulfate decreased 50.8% after 35 days, and 47.8% during 65 days, and 48.4%, at the end of 95 days. The falls of the serum AST content in exposed Nile tilapia were 39% in 35 days period, 41% in 65 days 44.2% in 95 days period whereas the ALP values elevated 20.1% during 35 days period, 23.1% after 65 days period and 26.2% in 95 days period.

In our study, the serum calcium concentrations in the fish group were exposed to $CuSO_4$.(5 H_2O) decreased 7% during 35 days, 8.3% after 65 days and 9.7% in 95 days period. A decrease in calcium value of serum might be caused from a deficit in calcium content of tissues and the increased excretion of calcium. Our results differed from Chen et al. (Chen *et al*., 2004), who reported an insignificant elevation in calcium concentration during the course of copper sulfate exposure in short-term; however, they used lower dosage of copper for shorter time than we did in our study. Calcium concentrations in teleosts are normally well regulated (Chen *et al*., 2004). Due to protein binding, calcium levels vary with total protein.

In 35, 65 and 95 days periods, serum phosphate concentrations of the Nile tilapia were exposed to $CuSO_4$.($5H_2O$) groups declined by 34.9%, 33.5% and 45.3%, respectively. The explanation for a decrease in phosphate value of blood was probably that the phosphate released by the bones was reused in phospholipids, nucleoprotein and nucleotide synthesis to counter the stress condition. Hypocalcaemia associated with decreased phosphate concentration was demonstrated by John (John, 2007). This was likely to be a result of kidney dysfunction.

The serum magnesium content of tilapia might drop with the effect of copper sulfate (Chen *et al*., 2004) as the decreases in the Nile tilapia exposed to copper sulfate were 32% during 35 days, 29% after 65 days and 30.3% at the end of 95 days period in our study. It is known that magnesium has the roles such

as neuromuscular conduct, activity of enzymes and hormones, peripheric function and regulation of metabolism of sodium and potassium in cells in mammals, but further fish studies are needed.

The decreases in the serum iron concentrations of the Nile tilapia exposed to copper sulfate, which showed an increasing percentage trend from short- to long-term, were observed 38.6% in 35 days, 59.3% in 65 days and 70% in 95 days period. Exposure of copper can cause a decrease of iron in serum/plasma offish (Chen *et al*., 2004). In this study, the decreases observed in serum Fe levels of 35-, 65- and 95-day trial periods in proportion to control group have been found to be similar with the findings of Chen *et al* (2004).

The decreases of serum potassium levels in the Nile tilapia exposed to $CuSO_4$.(5 H_2O) were 36.6% after 35 days, 29.9% during 65 days and 39.1% following 95 days. The positive correlations were observed between potassium and serum proteins, except total protein. A significant reduction of the blood serum potassium concentration produces a rise of the gradient of this cation between tissue and extracellular fluids, which can lead to an increase in the membrane potential of body cells. Decreased serum/plasma potassium (Gopal *et al*., 1997), calcium, phosphate (John, 2007) and magnesium levels in fish could be attributed to kidney damage.

The decreases of serum chloride concentrations in the exposed Nile tilapia were 23.7% in 35 days, 16% in 65 days and 22.9% in 95 days. Similarly, the serum sodium contents decreased in the Nile tilapia exposed to $CuSO_4$ ($5H_2O$) by 33% in 35 days, 23.7% in 65 days and 26.7% in 95 days. Serum sodium and chloride levels in fish could be reduced due to sodium and chloride uptake inhibited by copper as previously reported (Mazon *et al*., 2002). Concentrations of sodium and chloride are regulated by chloride cells located in gill epithelium. Copper could have an adverse impact on osmoregulation in freshwater fish (Mazon *et al*., 2002; Schjolden *et al*., 2007; Chen *et al*., 2004); exposed fish exhibits decreases in serum electrolytes, with an apparent inhibition of sodium and chloride uptake in gill, like it was observed in our study. Exposure to copper sulfate might affect ionoregulation of the Nile tilapia by depressing serum magnesium, potassium, sodium, iron, phosphate and calcium. The role of copper sulfate in ionoregulatory disturbance, mainly indicated by reduced serum electrolyte concentrations, has been well established. There was neither mortality nor any clinical signs although serum ions decreased to 28.5% chloride, 36.6% potassium, 38% magnesium, 33% sodium, 34.9% phosphate, 7% calcium and 38.6% iron in 35 days of exposure. It is concluded that the Nile tilapia can, despite significant reductions of blood ions, survive long (for 35 days) without mortality and gross pathological signs. However, gross clinical signs, which were necrotic pale gills and liver, with decreases of serum ions were observed in the exposed Nile tilapia at the end of both 65 and 95 days periods.

It is concluded that low-level copper sulfate exposure did not cause any damages in the Nile tilapia, because any gross pathological symptom such as anorexia, behavioral abnormality, external or internal clinical signs were not observed; although glucose, hepatic enzymes, proteins, nitrogenous compounds, lipids and ions in blood did significantly change for 35 days period. However, low-level copper sulfate lead to the changes of blood chemistry, damages of gills, liver and spleen in fish after 65 and 95 days of exposure. As a result, further studies including histological examinations should be carried out.

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