Effect of β-glucan on behavioral, biochemical and hematological parameters against toxicity of copper sulfate in common carp *Cyprinus carpio* L.

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Summary

The aim of the present study was to assess the effect of a commercial ß-glucan against acute toxic effect of copper sulfate on common carp *Cyprinus carpio* L. behavioral, hematological parameters and biochemical tests. A total of 250 Fish (150 ± 2 g) were fed a basal control diet or the basal diet plus a ß-glucan (1 g kg^{-1} diet) for 60 days. LC50 was calculated for 72 h which was 10.83 mg L^{-1}. At the end of the trial, $CuSO_4.5H_2O$ was added to all treatments at a dose of 5 mgL^{-1} for T1 and T2, 7mg L^{-1} for T3 and T4, 9 mgL^{-1} for T5 and T6 for 96h. Fish behavioral changes were recorded during 96h of exposure to different Cu concentrations with and without β-glucan, which showed abnormalities such as increase swimming activity, jerky movement, loss of equilibrium, respiratory stress, increase operculum movement, frequent jumping, erratic swimming and swimming near the water surface. On the other hand, ß-glucan supplemented groups showed significant increase (P≤0.05) in Hb concentration, PCV%, RBC and WBC count in comparison with groups without ß-glucan. The results of dietary groups without ß-glucan showed a significant decrease (P≤ 0.05) in Hb concentration, hematocrit value, RBC count, but there were significant increase (P≤ 0.05) in WBC count with an increase of Cu concentration compared to control groups. Results showed significant increase (P≤ 0.05) in dietary groups without ß-glucan in values of ALT, AST and ALP enzymes activity with elevation of Cu levels compared to control group. But, ß-glucan supplemented groups showed significant decrease (P ≤ 0.05) in values of ALT, AST and ALP enzymes activity in comparison with dietary groups without ß-glucan. These results suggested that β-glucan has protective effect against $CuSO_4$ toxicity and future studies should be pursued particularly immune response and molecular studies.

Keywords: β-glucan, *Cyprinus carpio*, $CuSO_4$, Hematology.

Introduction

The global production of farmed fish shows a rapid increase (18% per year) in production and (17.8% per year) of the aquaculture activities during 1997 to 2009. Hence, world aquaculture farming has more than doubled in the past 15 years (1). Furthermore, the increasing pollution of aquatic ecosystems with thousands of anthropogenic and natural chemicals is becoming the major environmental threat facing human and environmental health (2). In recent years, significant attention has been paid to the problems of environment contamination by pathogenic microbes and organisms (bacteria, viruses, and parasites), harmful algal blooms, increased ultraviolet (UV) radiation, and nutrient enhancement or deprivation (3). The main source of freshwater pollution can be attributed to discharge of untreated waste, which means in intensive method, decrease of water quality, increase of stress, decrease of food quality, increase bacterial, viral or parasitic infections that have negative effects on aquatic (4). Whilst copper (Cu) is essential for several fish metabolic functions. It is also used as fungicide, algaecide and herbicide (a chemotherapeutic agent) and in municipal water treatment systems. However, it is toxic at elevated concentrations for aquatic life (5). World production of Cu has increased in the last few decades and contamination by Cu has become increasingly prevalent in the aquatic environment (6) which is likely to increase bearing in mind manufacture and disposal of wide varieties of Cu-based products including agrochemicals. The application of chemicals to pond culture is also quite expensive and undesirable because of its risk of environment and culture contamination as well as impairing the growth of fish (7). Therefore, instead of

chemotherapeutic agents, increasing attention is being paid using prebiotic, probiotic and for diseases control measures in aquaculture (7) the application of probiotic and prebiotic in aquaculture have shown positive results. The prebiotics have several advantages over probiotics, they are natural feed ingredients, their incorporation in the diet does not require particular precautions and their authorization as feed additives may be more easily obtained, in spite of some concerns about their safety and efficacy. Originally, prebiotics were chosen to stimulate bifidobacteria and lactobacilli in human microbiota (8).

On the backdrop of above information, few studies have addressed the combination of prebiotics (β-glucan) and toxicity of waterborne Cu, regardless of waterborne being an important route of contamination in wild fish (9). Also, there are still many gaps in the information of Cu toxicity in fish. Therefore, the objective of the present study was to assess the effect of a commercial ß-glucan on behavioral changes, biochemical and hematological parameters against acute toxicity of copper sulfate in *Cyprinus carpio*.

Materials and Methods

Dietary preparations: Two diets were formulated using the same basal ingredients (Table, 1). This basal diet was used for the control group and ß-glucan (Schering-Plough Aquaculture, UK) was added at 1 gkg^{-1} to the basal mixture (manufacturer's recommended inclusion level), at the expense of cornstarch, to produce the ß-glucan diet. Dietary ingredients were mixed in a food mixer (model 4, Thoms-Wiley Laboratory Mill, USA) with warm water until a soft slightly moist consistency was achieved. This was then cold-press extruded (model P6; La Monferrina, Asti, Italy) to produce a 2-mm pellet.

Table, 1: Dietary formulations and proximate composition.

Ingredients %	Control diet	ß-glucan diet
Animal protein **	10 %	10 %
Soybean cake	40 %	40 %
Ground yellow corn	35%	34.9%
Corn oil	3 %	3 %
Wheat bran	9.5 %	9.5 %
Starch as binder	2.5 %	2.5 %
β- glucan	-	0.1%

****Animal protein obtained from al Hait Jordan Company contained 50 % protein, 2800 Cal kg^{-1}, 12% lipid, 25% ash, 2.9% calcium, 1.75% phosphor, 2.55% methionine, 2.8% cysteine.**

Experimental design: Common carp *Cyprinus carpio* were obtained from a carp farm at Al- Jadeda Diyala, Iraq. Fish were transported to the Aquaculture and Fish Nutrition Research Aquarium, College of Veterinary Medicine/ University of Baghdad. After two weeks acclimation and on growing 250 fish (150± 2 g) were randomly distributed into 12 trough tanks (150 x80 x 50 cm) filled with chlorine free tap water. Ten fish per trough, two replicates were maintained for each of the eight treatments. First control (C1), T1, T3 and T5 were maintained on diet without β- glucan , second control (C2), T2, T4 and T6 were maintained on diet with β–glucan. Fish were fed either the basal diet or the β–glucan diet at 3% biomass per day for 60 days. Water temperature was maintained at 22-25^{o}C, pH was 6.4-8.6 and dissolved oxygen maintained 6.-4-7.5 mg L^{-1}. A 12-h light/ 12-h dark photoperiod was maintained throughout the trial. Tanks were cleaned every day and water was changed. After determination of LC_{50} for $CuSO_4.5H_2O$ (Bio-Green Cut), $CuSO_4.5H_2O$ was added to all treatments at a concentration of 5 mgL^{-1} for T1 and T2, 7mg L^{-1} for T3 and T4, 9 mgL^{-1} for T5 and T6 for 96h. In the fifth day blood samples were collected to evaluate hematological parameters and enzymes activity. Behavioral changes of fish were described also.

Determination of median lethal concentration (LC_{50}) of copper sulfate: A pilot study was carried out to determine the median lethal concentration (LC_{50}) of copper sulfate. Five treatments of ten fish each were

transferred to bath trough and maintained for twoweeks acclimation and feeding was suspended 24 hours before the beginning of the experiment. Control group of fish was also established. Five copper sulfate concentrations were used. The concentration at which 50 % mortality of fish occurred after 72h was selected as the medium lethal concentration (LC_{50}). The LC_{50} was calculated by the probit analysis method. In this study, the observation of symptoms such as movement, respiration, swimming, feed intake and response to external stimuli was also recorded. The concentrations used in this experiment were 5, 7, 9, 11 and 13 mg L^{-1} respectively.

Biochemical analysis: Colorimetric determination of Alanine aminotransferase activity (ALT), Alkanline phosphatase enzyme (ALP) activity and Aspartate aminotransferase activity (AST) were performed according to (10).

Haematological parameters: Blood was sampled from six fish per tank after 96 h exposures to Cu. Samples were taken from the caudal vein using a 25 gauge needle and 1-ml syringe. Haematocrit (measured and read as % packed cell volume [PCV %]), haemoglobin, erthyrocyte counts (RBC), leucocyte counts were determined according to standard methods as described elsewhere (11). Statistical analysis was performed using SigmaPlot v11.0 software. All data were presented as mean ± standard error and analysed using one way analysis of variance (ANOVA) or Kruskal Wallis test, followed by multiple range tests. P values < 0.05 were considered significant.

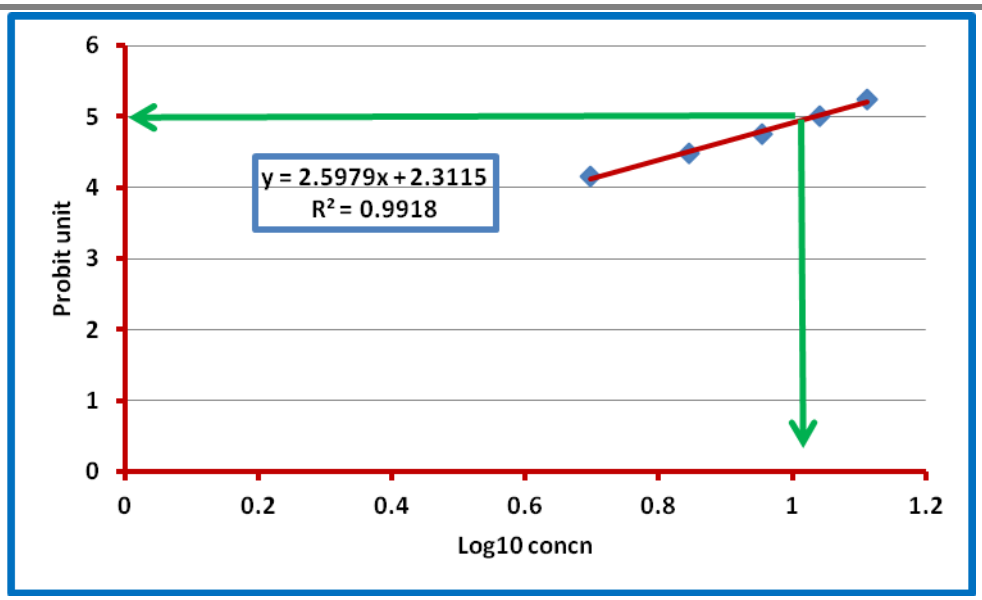
Results and Discussion

Determination of LC_{50}: In acute toxicity test of exposure to different sublethal concentrations of $CuSO_4.5H_2O$ after 72 h, LC_{50} was estimated by probit method (Table, 2). Fish showed abnormal behavior such as, Jerky movements, frequent jumping, increased swimming activity, fish surfaced frequently to gulp air, increased operculum movement, loss of equilibrium and erratic swimming. Also, fish was sluggish with slow or no response to stimuli after that they sunk to the bottom of water and die. The effect of acute toxicity of Cu at different concentrations on *C. carpio* is shown in (Figure, 1).

The present study showed that 10.83 mg L^{-1} is the LC_{50} of Cu during 72h of exposure. Mortality of the fish indicated that the toxicity of Cu increased with the increase of its concentration. Results showed that no mortality of fish in the control group but in another study, 96 h LC50 values of $CuSO_4$ for adult tilapia *Oreochromis niloticus* and catfish *Clarias gariepinus* were recorded to be 58.83 mg L^{-1} and 70.13 mgL^{-1} respectively (12). Also (13) recorded that 48h. LC_{50} value of CuSO4 for juvenile channel catfish *Ictalurus punctatus* was 28 mgL^{-1}. Another study, 96 h LC_{50} value of copper was found to be 5.5 mgL^{-1} in fingerlings of *Esomus danricus*, (13). The differences in the toxicity values of copper might be due to the physicochemical characteristics of the test medium, species and ages of fishes used and their susceptibility rates, which resulted in their subsequent toxicity values (14).

Table, 2: LC_{50} of $CuSO_4.5H_2O$ toxicity in *C .carpio* measured by probit method

Conc. mgL^{-1}	Fish No.	Mortality No.	Survival %	Log10 conc.	Probit unit
0	10	0	10	-	-
5	10	2	8	0.698	4.16
7	10	3	7	0.845	4.48
9	10	4	6	0.954	4.75
11	10	5	5	1.041	5.00
13	10	6	4	1.113	5.25



Figure, 1: Linear relationship between probit response and log10 concentration of LC_{50} of $CuSO_4.5H_2O$ for 72h in *C. carpio*.

Behavioral changes: Fish showed behavioral changes after exposure to the different Cu concentrations such as jerky movement, frequent jumping, and increase swimming activity. After period of stressful avoidance through various behavioral anomalies, fish remained suspended in vertical position with the mouth up near the water surface and the tail pointing downward with increase operculum movement, fish lost its equilibrium and had erratic swimming. Fish was dull with no response to stimuli and became motionless with increased of mucous production; finally, it sank to the bottom of water and died. The severity of sings increased with the increased of Cu concentrations. The addition of β- glucan reduced the severity of behavioral changes to be less than that of copper alone. These results are in agreement with (15) who recorded that Cu concentration on some fish species lead to damage of the chemoreceptors and mechanoreceptors in the lateral line. Cu caused abnormal behavior in exposed fish because it induces lesions in the olfactory organ and lateral line (16). Since the lateral line was considered the key organs in the mediation of fish behavior and it is important for detection and localization of prey, enemy avoidance, schooling and intraspecific communication (17). Thus, any substance that causes irritation or lesions to the lateral line may adversely affect the normal behavioral pattern of fish. Furthermore, change in olfactory function induced by a toxicant may affect the normal adaptive response of fish (18). The decrease in fish activity may imply reduce metabolism as the concentration of coppr increases. This agrees with (19) who observed that copper concentration and exposure duration were dependent on the decrease in the locomotors activity of the bluegill *Lepomis macrochirus* exposed to 0.04, 0.08 and 0.4 mgL^{-1} Cu. Loss of equilibrium might be due to the inhibition of brain cytochrome C oxidase activity, causing cytotoxic hypoxia, thus causing brain damage to the region of the brain associated with the maintenance of equilibrium (20). Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which might be due to accumulation of acetylcholine in synaptic and neuromuscular junctions (21).

Haematological parameters: The results of the various indices for the treatment are summarized in (Table, 3). There are significant increases ($P\leq 0.05$) in RBC_S count, PCV % and Hb concentration (gdl^{-1}) in C2 in comparison with C1. However, there were significant decreases ($P\leq 0.05$) in RBC_S, PCV and Hb of T2 in comparison with C1, followed by significant decrease ($P\leq 0.05$) in RBC_S count, PCV and Hb compared to C1 and there were significant difference ($P\leq 0.05$) between T1 and T2. Also, T3 showed a significant decrease ($P\leq 0.05$) in RBC_S, PCV and Hb of in

comparison with C1 and also there were significant differences (P≤ 0.05) between T3 and T4. Additionally, there is a significant decrease (P≤ 0.05) in RBCs, PCV and Hb of T6 followed by T5 in comparison with C1. Also there are significant differences (P≤ 0.05) between T5 and T6. These results are in line with (22) who found that exposure of fish *Channa punctatus* to copper sulphate showed a significant decrease in RBCs from 2.86 to 1.84x 10^6 cell /mm^3, PCV from 31.00 to 23.33 % and Hb content from 10.73 to 6.60 gdl^{-1} as compared to control. (23 and 24) found a significant reduction of RBCs, PCV and Hb in fishes exposed to different heavy metals.The reduction in RBCs, PCV and Hb content may be due to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sub-lethal concentration of pollutants. While (25) suggested that heavy metal exposure decreased the red blood cell count, PCV and Hb due to impaired intestinal absorption of iron. The reduction in RBC count might be due to the destruction of mature RBCs and the inhibition of erythrocyte production (26). According to (27) the reduction in Hb content in fish exposed to toxicant could also be due to the inhibitory effect of the toxic substances on the enzyme system responsible for synthesis of Hb. The obtained result showed that the prebiotic (β-glucan) could be served as substrate for growth and proliferation of anaerobic bacteria mainly the bifidbacteria present in caeco colon (28). These bacteria can be enhanced the metabolism and increase the vitality of cells by supplying oxygen to whole body particularly heamopoietic center. Also have the ability to produce a lot of essential vitamin B complex members particularly biotin and vitamin B_{12} that resulted in high food utilization specific protei, iron and cobalt from diet intake that were essential members for red blood cell producing (29).

Results for WBC counts showed a significant increase (P≤ 0.05) in WBCs count of C2, T1 and T2 in comparison with C1. Also, there was a significant difference (P≤ 0.05) between T1 and T2 which recorded highest value. However, there were no difference between C2 and T1, C2 and T2. Additionally, a significant increase (P≤ 0.05) was observed in WBCs counts of C2, T3 and T4 in comparison with C1. Also there were significant variations (P≤ 0.05) between C2 and T4 and between T3 and T4 which were recorded the highest value. Statistical analysis showed a significant increase (P≤ 0.05) in WBCs count of C2, T5 and T6 which were compared to C1. Moreover, there were significant variations (P≤ 0.05) between C2 and T5 and between C2 and T6 which recorded the highest group. These results are in agreement with (30) who found increased WBC count (41 .24 x 10^3 cell ml^{-1}) on *Oreochromis niloticus* post β- glucan treatment compared with control group (35.76 x10^3 cell ml^{-1}). (31) Reported elevation total leukocytes count in *C. carpio* injected with β– glucan.

Table, 3: Hematological parameters of *C . carpio* which exposed to different concentrations of Cu with and without β-glucan

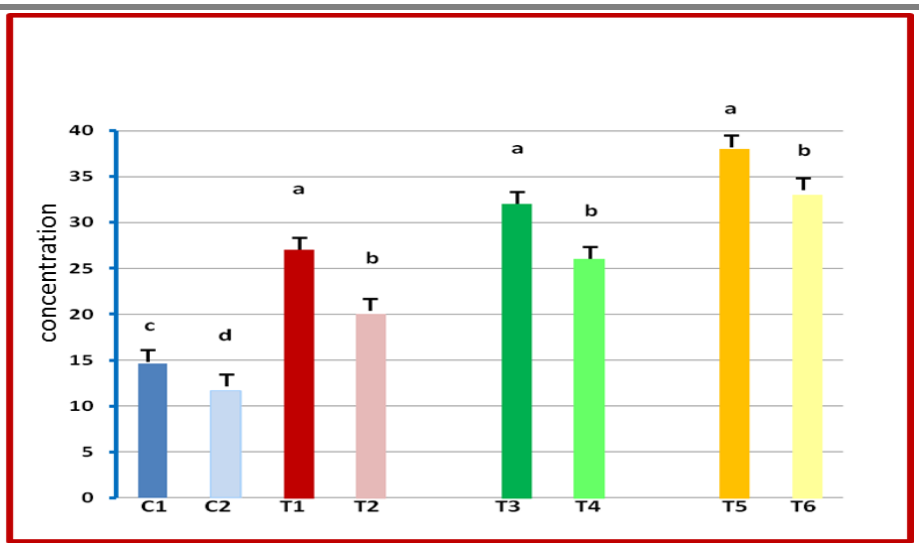
Treatment.	RBC x10^6/mm^3	PCV%	Hb g/dl	WBC x10^3/mm^3
C1	2.30±0.08 b	28.00±0.28 b	8.00±0.28 b	16.00±0.28 c
C2	3.00±0.07 a	31.00±0.57 a	10.00±0.27 a	20.00±1.52 ab
T1	1.40±0.02 d	23.00±0.28 d	5.50±0.50 d	18.00±0.38 b
T2	1.85±0.08 c	26.00±1.04 c	7.00±0.29 c	21.50±0.27 a
T3	1.25±0.07 d	21.00±0.57 d	5.00±0.57 d	20.00±1.66 b
T4	1.55±0.07 c	24.00±0.28 c	6.50±0.28 c	23.00±0.86 a
T5	1.10±0.05 d	18.00±0.57 d	4.00±0.28 d	22.50±0.70 b
T6	1.35±0.05 c	20.00±0.57 c	5.30±0.57 c	25.00±1.01 a

Values are expressed as mean ± SE, means having the different letters in the same column are significantly different at (P ≤ 0.05).

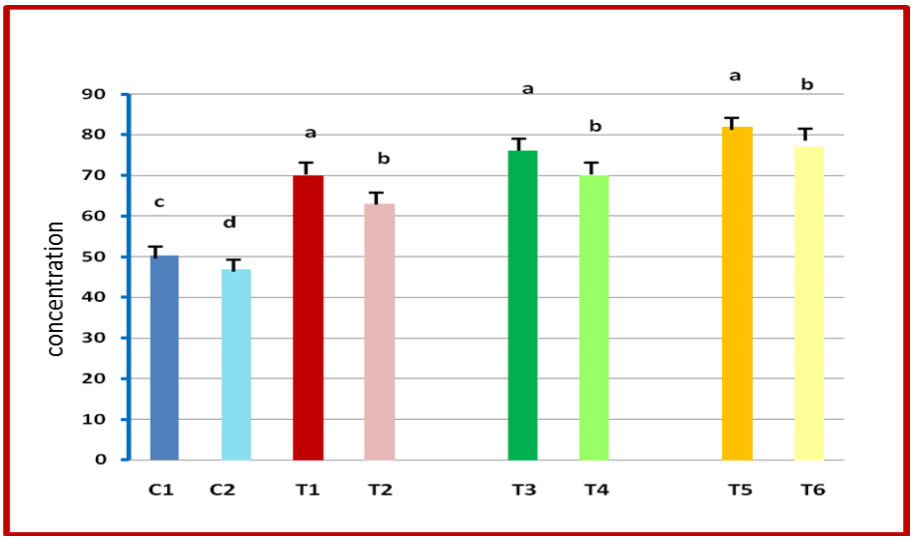
Results in serum ALT (IU/L) was observed a significant decrease (P ≤ 0.05) in C2 (11.66 IUL^{-1}) in comparison with C1 (14.66 IUL^{-1}), while there are significant increase (P≤ 0.05) in ALT activity of T1and T2 (27 and 20 IUL^{-1}) respectively in comparison with

C1. Also there were significant differences (P≤ 0.05) between T1 and T2. The results also showed a significant increase (p≤ 0.05) in the ALT activity of T3 and T4 (32 and 26 IUL^{-1}) respectively in comparison with C1. In addition to that, there were significant differences (P≤ 0.05) between T3 and T4, and a significant increase (P≤ 0.05) in ALT of T5 and T6 (38 and 33 IUL^{-1}) respectively compared to C1 (Figure, 2). In contrast, AST showed a significant decrease (P ≤ 0.05) in C2 (46.8 IU/L) in comparison with C1 (50.3 IUL^{-1}), but there was a significant increase (P≤ 0.05) in T1 and T2 (70 and 63 IU/L) respectively compared to C1 and there were significant differences (P≤ 0.05) between T1 and T2. Also, there were significant increase (P≤ 0.05) in AST of T3, T4 (76 and 70 IUL^{-1}) respectively in comparison with C1. While significant differences (P≤ 0.05) were recorded between T3 and T4. There were also significant increase (P≤ 0.05) of T5, T6 (82 and 77 IUL^{-1}) respectively in comparison with C1 (Figure, 3). Concerning serum ALP activity (IUL^{-1}) there was a significant decrease (P ≤ 0.05) in ALP of C2 (36.33 IU/L) in comparison with C1 (39.5 IUL^{-1}). However, a significant increase (P≤ 0.05) of T1, T2 (52 and 45 IUL^{-1}) respectively compared to C1. While there were significant differences (P≤ 0.05) between T1 and T2. In addition to that, there was a significant increase (P≤ 0.05) in ALP of T3, T4 (57.83 and 51 IU/PL^{-1}) respectively in comparison with C1. While there were significant differences (P≤ 0.05) between T3 and T4. The results showed also a significant increase (P≤ 0.05) in ALP of T5, T6 (63 and 57 IUL^{-1}) respectively in comparison with C1 while there were significant differences (P≤ 0.05) between T5 and T6 (Figure, 4). These results are in agreement with (32) who found that serum ALT , AST and ALP activities of *O. niloticus* increased in response to Cu and Pb exposure when compared to control during 4 and 21 days. While (33) found that sub-lethal concentration of cadmium caused a significant increase in ALT, AST and ALP of *C. carp*io after 7 and 25 days. On the other hand, (29) showed that both ALT and AST enzymes were increased significantly after exposure of fingerlings of *O.niloticus* to sub-lethal concentrations of either copper or lead, serum AST values recorded after 4 weeks of exposure to Cu-polluted water (2 and 1 mgL^{-1}) and Pb-polluted water (12 and 6 mgL^{-1}) were 127, 59, 101 and 83 IUL^{-1} respectively. Serum ALT values recorded in the above mentioned treatments after 4 weeks of exposure were, 54, 103, 56 and 152 IU L^{-1} respectively. These results is in line with (29) who showed that ALT, AST and ALP activity were significantly decreased in the treated fish treatments with β- glucan in comparison with Aflatoxin B1 (AFB1) treated groups. These results also are agreement with (34) who found that the supplementation of live *Saccharomyces cerevisiae* (10g) (contain β-glucan) for Galilee tilapia *Sarotherodon galilaeus* L. for 6 weeks have reduced copper absorption and accumulation in fish body and improved its growth.

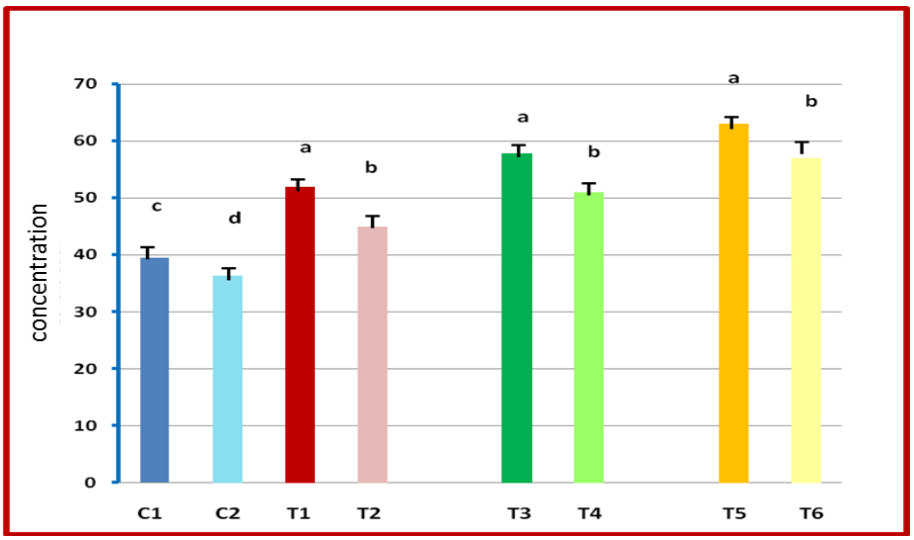
Serum ALT, AST and ALP activity were increased significantly after copper exposure which may indicate hepatocellular damage or cellular degradation in liver, spleen or muscles (35) and become more permeable leading to some of these enzymes to leak out into the blood stream. Furthermore, an increase of these enzymes activities in the extracellular fluid or serum is a sensitive indicator of even minor cellular damage (36). It was observed that the exposure to heavy metals resulted an increase of ALT, AST and ALP activities in plasma/serum of fish *Sparus aurata* and *C. carpio* (37). The addition of prebiotic (β-glucan) decreased significantly serum ALT, AST and ALP activity to be lower than that of copper alone which could be due to that β – glucan have antioxidant properties (38 and 39) and the ability of β – glucan repair the damage of cells (40).



Figure, 2: ALT value of *C .carpio* which exposed to different concentrations of Cu with and without β-glucan after experimental period.



Figure, 3: AST value of *C. carpio* which exposed to different concentrations of copper sulfate with and without β-glucan after experiment period.



Figure, 4: ALP value of *C. carpio* which exposed to different concentrations of Cu with and without β-glucan after experimental period.

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تأثير البيتاكلوكان ضد سمية كبريتات النحاس في التغيرات السلوكيه و الكيموحيويه والصوره الدمويه في اسماك الكارب الشائع *Cyprinus carpio* L.

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الخلاصه

هدفت الدراسة تقييم تأثير اضافة البيتا كلوكان في غذاء الاسماك ضد التسمم الحاد لكبريات النحاس وتقدير التسمم الحاد لكبريتات النحاس وذلك عن طريق دراسة التغيرات السلوكية و التغيرات الدموية والفحوصات الكيميوحيوية في اسماك الكارب الشائع *Cyprinus . carpio* استعمل في الدراسة 250 سمكة من اسماك الكارب الشائع معدل اوزانها 150± 2 غم وزعت على ستة معاملات مع معاملتين سيطرة . تم حساب التركيز المميت الوسطي خلال 72 ساعه اذ بلغ 10.83 ملغم / لتر. غذيت اسماك المعاملات C1 و T1و T3 وT5 على عليقة بدون اضافة بيتا كلوكا ن بينما المعاملات C2 و T2 وT4 وT6 اضيف اليها البيتا كلوكان(1غم \كغم) لمدة 60 يوما. استعملت ثلاث تراكيز من كبريتات النحاس 5 ملغم / لتر(T1 بدون بيتا كلوكان وT2 مع البيتا كلوكان) و 7 ملغم / لتر(T3 بدون بيتا كلوكان وT4 مع البيتا كلوكان) و 9 ملغم / لتر (T5 بدون بيتا كلوكان و T6 مع البيتا كلوكان) لمدة 96 ساعة. سجلت التغيرات في سلوك الاسماك خلال 96 ساعة من التعرض لتراكيز مختلفة من كبريتات النحاس بوجود وعدم وجود البيتا كلوكان, اذ اختلفت شدتها حسب التركيز وتمثلت بزيادة حركة الاسماك وحركة متقطعة و فقدان توازن و صعوبة التنفس و زياده حركة الغطاء الغلصمي والقفز المتكرر والسباحة بصورةغير طبيعية والسباحة قرب سطح الماء. اظهرت نتائج المعايير الدموية زياده معنويه ($P\leq 0.05$) في تركيز الهيموغلوبين و حجم خلايا الدم المرصوصة وخلايا الدم الحمر والبيض في مجاميع البيتاكلوكان مقارنة بمجموعة السيطرة كما اظهرت نتائج المجاميع بدون اضافة البيتاكلوكان انخفاض معنوي ($P> 0.05$) في تركيز الهيموغلوبين و حجم خلايا الدم المرصوصة وخلايا الدم الحمر مقارنة بمجموعة السيطره بزياده تركيز النحاس. بينما اظهرت كريات الدم البيض زياده معنويه ($P\leq 0.05$) بزيادة تركيز المادة السامة مقارنة بمجموعة السيطرة. اظهرت دراسة الفحوصات الكيموحيوية في المجاميع بدون اضافة البيتاكلوكان وجود ارتفاع معنوي ($P\leq 0.05$) في قيم انزيمات الكبد ALT و AST وALP بزيادة تركيز المادة السامة مقارنة بمجموعة السيطرة بينما اظهرت مجاميع البيتاكلوكان انخفاض معنوي ($P> 0.05$) في قيم انزيمات الكبد ALT و AST وALP بزيادة تركيز المادة السامة مقارنة بمجموعة السيطرة. تستنتج هذه الدراسه الاهميه الوقائيه للبيتاكلوكان ضد التأثير السمي للنحاس واهميه اجراء دراسات مستقبليه لاسيما الاستجابه المناعيه والجزيئيه.

الكلمات المفتاحية: البيتاكلوكان، الكارب الشائع، كبريتات النحاس، الصورة الدموية.