EFFECT OF KAOLIN ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF *CYPRINUS CARPIO* L. AGAINST COPPER SULFATE TOXICITY

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ABSTRACT

The present study was aimed to evaluate the effect of kaolin on copper sulfate toxicity and on some hematological and biochemical performance in *Cyprinus carpio* L. 240 common carp ranged between 85-95g in weight. A total of 120 fish were used to determine the median lethal concentration LC_{50} of copper sulfate and 120 fish and randomly distributed into six treatments (two replications for each treatment). The LC_{50} of copper sulfate was 1.82 mg/l for 48h exposure, then added 0.91mg/l of copper sulfate to all treatment groups except control negative .The experimental treatments included adding kaolin: Control negative (without copper sulfate or kaolin); Control positive 0.91mg/l copper sulfate without kaolin.T1, T2, T3 and T4 kaolin were added in the water at levels of 2, 4 , 6 and 8 g/l respectively. Results showed that the values of RBCs count were ranged 1.16 $x10^6$ - 1.77$x10^6$ cell /mm^3 in cont.+ve and T3 respectively. WBCs count were ranged 8.40×10^3 in cont.+ve to 13.14×10^3 cell/mm^3 in T3.The lowest Hb content found in cont.+ve reached 8.35 g/dl, while the highest content found in control-ve attained 12.87g/dl. PCV ranged from 21.75 to 35.60% in cont.+ve and T3 respectively. Results of total protein ranged between 6.6 - 18.19 g/dl in T4 and cont.-ve respectively, while albumin ranged from 1.31 to 1.53 g/dl in cont.+ve and cont.-ve respectively. Globulin level recorded the lowest value in T4 which was 5.15g/dl whereas the highest level in cont.-ve which was 16.67 g/dl. In conclusion, this investigation indicated that kaolin has reduced the toxicity effects of copper sulfate in common carp and the best level can be use was 6g/l (T3).

Keywords: fish, blood, adsorption, total protein, diseases

مجلة العلوم الزراعية العراقية 2024-:55(عدد خاص):217-224 التميمي والرديني

تأثير الكاولين على الصفات الدمية والكيموحيوية لاسماك الكارب الشائع *Cyprinus carpio* L. ضد التسمم بكبريتات النحاس

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المستخلص

هدفت هذه الدراسة إلى تقييم تأثير الكاولين على سمية كبريتات النحاس وعلى بعض الصفات الدمية والكيموحيوية في اسماك الكارب الشائع استخدمت ٢٤٠ سمكة بوزن يتراوح بين 85–95غم، استخدمت منها 120سمكة لتحديد متوسط التركيز المميت LC_{50} من النحاس و 120 سمكة أخرى للعلاج بالكاولين. وزعت عشوائيا إلى ست معاملات (مكرران لكل معاملة). بلغ LC_{50} من النحاس 1,82 ملغم / لتر بفترة تعرض لـ ٤٨ساعة. ثم أضيف تركيز 0,91ملغم / لتر من النحاس الى جميع معاملات العلاج عدا مجموعة السيطرة السالبة. حيث كانت معاملات التجربة كالتالي: مجموعة السيطرة السالبة (دون النحاس أو الكاولين)؛ مجموعة السيطرة الموجبة (النحاس ٠،٩١ بدون كاولين) والمعاملات الأولى والثانية والثالثة والرابعة، تمت إضافة الكاولين في الماء بتراكيز 2 و 4 و 6 و 8 غم / لتر على التوالي. أظهرت النتائج أن قيم عدد كرات الدم الحمراء تراوحت بين 1,16 –1,77 ×١٠^{٦} خلية/ ملم ^{٣} في معاملة السيطرة الموجبة والمعاملة الثالثة على التوالي. وقد تراوحت تعداد الكريات البيضاء بين 8,40 – 13,14 × ١٠^{٣}خليه/ملم ^{٣}في مجموعة السيطرة الموجبة والمعاملة الثالثة .وجد أدنى محتوى للهيموجلوبين في مجموعة السيطرة الموجبة وصلت الى(8,35 غم / ديسيلتر) في حين عثر على أعلى نسبة(12,87 غم/ ديسيلتر) في مجموعة السيطرة السالبة. تراوحت نسبه الخلايا المرصوصة بين 21,75 –35,60 % في مجموعة السيطرة الموجبة والمعاملة الثالثة على التوالي. نتائج البروتين الكلي تراوحت بين 6,6 حتى 18,19 غم/ ديسيلتر في المعاملة الرابعة و مجموعة السيطرة السالبة على التوالي، في حين تراوح الألبومين بين 1,31 – 1,53غم / ديسيلتر في مجموعة السيطرة الموجبة و مجموعة السيطرة السالبة على التوالي. أظهرت النتائج أن أدنى قيمة للجلوبيولين كانت ٥،15 في المعاملة الرابعة واعلى قيمة كانت16,67غم/ديسيلتر في مجموعة السيطرة السالبة. يمكن الاستنتاج بأن الكاولين قد قلل من تأثير سمية كبريتات النحاس في الكارب الشائع وأفضل مستوى كان باستخدام تركيز 6 غم /لتر(المعاملة الثالثة).

الكلمات المفتاحية: اسماك، دم، امتزاز، البروتين الكلي، امراض

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INTRODUCTION

Aquaculture is described as the production of aquatic organisms, including fish, invertebrates, mollusks, and aquatic plants(5).Common carp is the most widely cultivated fish in the world, it has a high growth rate, continental habits, high fertility, flexible nature, ease of adaptation to artificial feeds, resistance to diseases and harsh environmental conditions(4, 5, 23). Fish health is an indicator of the pollution of the aquatic environment (6, 7, 26). Industrialization is a major catalyst for growth and urbanization, which release of various toxic chemicals, gases, solid wastes, and microbes of various types into our immediate environment—land, air, and water, water pollution is of particular interest because it has become a global issue (21,28).Heavy metals are important components of the aquatic environment, usually found in extremely low concentrations , heavy metals levels are too high in the area where domestic activities, mining activities, mechanical and electrical activities, cultivating activities are spread throughout the natural areas(31). Copper, is highly toxic metal that is often considered poisonous even at low concentrations, is responsible for numerous metabolic processes are required to biological processes, copper is also a catalytic co-factor, for a minimum of 12 major proteins, as well as 30 different enzymes (3).Heavy metals potentially accumulate in aquatic environment including water, sediments and fish, and subsequently are transferred to humans through the food chain(8). Copper is used in a wide range of industries, including electrical instruments and all thermal and electrical processes. Fungicides are made from copper sulfate pentahydrate, and some fungi can also adapt to high concentrations of copper ions(15). According to Thrall *et al*.(4,6,33), hematological and biochemical parameters of fish can be used to assess the organism's health, Clinical hematological examination is cheap and can be done with simple methods automatic hematological analyses of fish blood have recently been attempted as an option to manual methods (16,18,29). High red blood cell count is one parameter that ensures a better oxygen supply to tissues. RBC measured in the blood of *C. carpio* was found to be 0.33 to 2.95×10^6 cell/ mm³ (35). The number and percentage of WBCs in fish blood circulation vary greatly, even among nonspecific individuals under similar conditions. Hrubec *et al.*(20) presented reference WBC values in the interval of 2.15 to 15.47 $\times 10^3$ cell/mm³. The PCV, also known as hematocrit or HC, is the volume percentage (volume %) of red blood cells (RBC) in the blood measured as part of a blood test (27). in the case of *C. carpio*, different authors obtained a range between 14.0–44.0% (35). The liver is the primary factory for the production of proteins in the body, Blood serum plays an important role in the normal balance of the body and is a store of amino acids and a carrier for many nutrients that can't be transported without it. Protein is the most important organic material required for tissue construction and repair, and it also plays an important role in providing energy to fish (10). Kaolin is a clay mineral made up of kaolinite material that has been extensively used in a variety of technological uses. The waste from textile dyeing is toxic and alters the properties of the water body(36). Like Zeolites are microporous crystalline hydrated aluminosilicates that have many applications due to their biocompatibility, such as ion exchange and adsorption–desorption properties.(1, 2). Adsorption has been identified as a potentially useful technology for removing pollutants from wastewater, clay-based adsorbents, such as zeolite and clay-based (bentonite, kaolin and montmorillonite) are cost-effective(11`). The aimed of present study was to evaluate the effect of kaolin on copper sulfate toxicity and on some hematological and biochemical performance in *Cyprinus carpio*.

MATERIALS AND METHODS

Determination of median lethal concentration (LC_{50}) of copper sulfate

A total of 120 fish were used to determine the median lethal concentration LC_{50} of copper sulfate. Different concentrations of copper sulfate dissolved in distilled water were added to 50 liters of water volume in each treatment. Concentration of copper sulfate solution was: 2, 4, 6, 8, 10 and 12 mg/l, mortality was recorded at 24, 48, 72 and 96 hr. The experiments was carried out for a period of 96

hr. the number of dead fish was calculated every 24 hr. and removed immediately from glass aquariums .

Experimental design: The experiment was conducted for one month in the Fish Diseases Laboratory of the College of Veterinary Medicine/University of Baghdad, *Cyprinus carpio* were brought from Al-Mahawil hatchery, with a weight ranged between 85-95g. They were acclimated for two weeks after disinfected it from any external parasites. A total of 120 fish were randomly distributed to 12 glass aquariums with dimensions of 40 x 40 x 70 cm filled with 50 liters of water with air pumps to supply water with a high and constant level of dissolved oxygen, and periodic feeding twice a day at a rate of 3% of fish weight. Experiment was divided into six replicate groups, a negative and positive control group, and four treatments using concentrations of 2,4,6 and 8 g/l of pure white Iraqi kaolin clay finely ground. Add 0.91 mg/l of copper sulfate in all treatment groups except control negative. No amount of water was changed during the experiment period.

Blood collection: Blood samples were taken via caudal vein puncture from two fish chosen randomly for each treatment after anesthesia by using ground cloves solution at a concentration of 10% for 10 minutes. Blood samples were transferred to Eppendorf tubes that had been coated with lithium heparin that works as anticoagulant and used for determination of the hematological parameters (RBC and WBC count, determination of Hb content and PCV %).Both total erythrocyte and leukocyte counts done by diluting 20 µl of freshly collected whole blood sample with 0.98 ml of Dacies(12) fluid(10ml of 40% formaldehyde, 31.3g trisodium citrate, 1.0 g brilliant crystal blue dissolve in one liter of distilled water and filtered through 0.45 µm syringe filter). Direct counting using a microscope can be used to determine the number of cells in the chamber, and visually measurable cells can be counted differentially. The number of cells in the chamber is used to compute the concentration or density of the cells in the mixture from which the sample was taken. It is the number of cells in the chamber divided by the volume of the chamber, which is known at the outset. Cells on or near the top and left lines are counted, but those on or near the right or bottom lines are not. A drop was dripped on the haemocytometer, five squares were used to count the red blood cells number (12). The followed equation was applied:Total RBCs (Erythrocyte / μL^6) = N (number of cell) x 2500. Total WBCs (Leukocyte / μL^3) = N (number of cell) x 125. Concent of Hb was determined by using the standard cyanomethemoglobin method described by Dacie and Lewis(8), were stored in a borosilicate glass bottle. The assay was achieved in test tubes where 20 µl of freshly collected blood was mixed with (5 ml) of diluents. The solution was inverted several times before being allowed to incubate at room temperature (25°C) for 10 min. Absorbance was measured using a Flam Atomic Absorption Spectrophotometer at 540 nm. PCV was computed using Klontz's method (24) . The hematocrit tubes were filled with blood and the bottom was sealed with sealant. Tubes were spun at high speed for 3 to 5 minutes in a micro hematocrit centrifuge(3000 gx). To calculate the hematocrit, a hematocrit reader measures the length of the packed red cell column and divides it by the length of the entire blood column (cells and plasma).

Biochemical profile: Biuret method was used for examination through a test that described by Weichselbaum (34).Total protein = (absorbance of samples/ absorbents of standards) × ng/dl: n=6, g/l: n=60. Measurement of albumin carried out according to Dumas and Biggs(14). Albumin (g/l)=(absorbers of samples /observance office standards)× Concentration standard 46.3g/l. Globulin was measured through removal of albumin value from total proteins value (13).

Statistical analysis: The Statistical Analysis System- SAS (30) program was used to detect the effect of different factors (Treatments and Period) in study parameters. Least significant difference –LSD test (2 way of Anova) were used to study the significant differences compare between means in this study.

RESULTS AND DISCUSSION

Determination of median lethal concentration (LC_{50}) of copper sulfate: Median lethal concentration measurement of copper sulfate was estimated LC_{50} by (Probit

analysis) according to Goldstein *et al*. (14) of copper sulfate exposure to *C. carpio*. Results of mortality rate of studied fish exposed to copper sulfate showed in an increase with increased copper sulfate concentrations (Tab.1). Table 2 showed LC_{50} in 48h exposure is 1.82 mg/ l.

Table 1. Total mortality of fish in 24,48,72 and 96h.with different concentrations of $CuSO_4$

No.	Total fish No.	$CuSO_4$ Con.(Mg/l)	Dead % in 24 hr	Dead% in 48 hr	Dead% in 72h	Dead% in 96 hr
1	10	2	0	0	0	0
2	10	4	33	50	83	83
3	10	6	17	50	66	100
4	10	8	50	10	100	100
5	10	10	66	100	100	100
6	10	12	50	83	100	100

Table 2. Mortality rate of fish after 48 hours

No.	Conc.(Mg/l)	Log10	Total fish	Dead fish	%	Probit Unit	LC_{50}	Log LC_{50}	Regression equation
1	2	0.30	10	0	0	0	45.63	1.65	Y=0.1255 X +0.2724
2	4	0.60	10	3.3	33	4.56			
3	6	0.77	10	1.7	17	4.05			R^2 =0.7701
4	8	0.90	10	5	50	5.00			
5	10	1.00	10	6.6	66	5.41			
6	12	1.08	10	5	50	5.00			

Al- Tamimi and Al-Azzawi (9) found that the median lethal concentration of copper sulfate was 3.36 mg /l for common carp at a rate of 35 g weight when exposed to copper sulfate toxicity for 48 hours. Another study in which the median lethal concentration of copper was determined on Amur carp *Cyprinus carpio haematopterus* fish with a weight ranging between 8-9 g for a period of exposure for 96 hours, which amounted to 1.81 mg/l (19).

Blood parameters

Results of present study showed that the RBCs count were ranged between 1.16 to 1.77 $\times 10^6$ cell/mm³ in control-ve and T3 respectively (Tab.3). The statistical analysis showed that the significant increase ($P\leq 0.05$)in T3 compared with other treatment groups. Also significant increase ($P\leq 0.05$) in T2 compared with T1 and T4. Control+ve was recorded a significant decrease ($P\leq 0.05$) compared with all groups of treatment. No significant different ($P> 0.05$) between control-ve and T3 WBCs count were ranged between 8.4 to 13.14 $\times 10^3$cells/mm³ in control +ve and T3 respectively. Table 3 showed a significant increase ($P\leq 0.05$) in T3 compared with other treatments groups, and significant increase ($P\leq 0.05$)in T2 and control-ve compared with T1,T4 and control +ve, also significant increase($P\leq 0.05$) in T1 compared with T4 and control +ve. No significant different ($P> 0.05$) observed between control-ve and T2. Hb content was ranged between 8.35 to 12.87 g/dl in control+ve and control-ve respectively. Hb content showed an increase with concentrations of kaolin up to T3 except T4 (Tab.3). There is no significant difference ($P>0.05$) between control-ve and T3. Whereas a significant increase ($P\leq 0.05$) observed in control-ve and T3 compared with other treatment groups, also there is a significant increase ($P\leq 0.05$) in T2 compared with T1, T4 and control +ve respectively. A significant increase ($P\leq 0.05$) has been found in T1 compared with control +ve and T4,whereas a significant decrease ($P\leq 0.05$)in control +ve compared with T4.The PCV value are ranged between 21.75 % in control+ve to 35.6 % in T3. A significant increase ($P\leq 0.05$) showed in T3 compared with other treatment groups, also between T2 and control-ve compared with T1, T4 and control +ve respectively. A significant increase ($P\leq 0.05$) observed in T1 compared with T4 and control +ve. While T4 showed significant increased ($P\leq 0.05$) compared with control +ve.(Tab.3).

Table 3. Values of hematological parameters (Mean ±SE) of fish exposed to copper sulfate and treated with different concentrations of kaolin during experimental period

Treatment	RBC×10^6count	WBC×10^3count	Hb content	PCV %
Control -ve	1.74 ±0.02 a	11.74 ±0.09 b	12.87 ±0.06 a	32.50 ±0.10 b
Control +ve	1.16 ±0.03 d	8.40 ±0.24 e	8.35 ±0.04 e	21.75 ±0.15 e
T1	1.48 ±0.04 c	11.10 ±0.05 c	10.64 ±0.07 c	30.00 ±0.80 c
T2	1.64 ±0.01 b	11.79 ±0.04 b	11.90 ±0.10 b	32.59 ±0.50 b
T3	1.77 ±0.04 a	13.14 ±0.05 a	12.83 ±0.08 a	35.60 ±0.09 a
T4	1.41 ±0.01 c	10.16 ±0.03 d	10.21 ±0.02 d	26.21 ±0.02 d
LSD value	0.0866 *	0.394 *	0.209 *	1.471 *

Means having different alphabets in columns are significant difference (P≤ 0.05)

Including a significant differences in red blood cell count and Hb content, this fact can be explained by a compensatory effect in relation to oxygen transport capacity (25). These alterations are due to the damage that copper sulfate causes in the gills and hematopoietic organs. The significant proportion of blood cell changes manifests as interference with ionic status and fluid volume (32). As a result of this toxicity, which led to a decrease in the red blood cells count and hemoglobin in the positive control group, the effect of kaolin concentrations was observed in changing the levels of blood parameters and their direction towards normal levels of PCV and Hb (27).The reason for this can be attributed to the kaolin adsorption ability of copper ions dissolved in water, as it has a highly efficient adsorbing, reducing the toxic effect of copper sulfate. The WBCs count was increased with increased kaolin concentration up to T3. Fink and Salibian (17) demonstrated that an increase in WBCs could be due to the induced production (as a result of chemical toxicity) of multipotent hematopoietic cells, which could be due to circulating WBC depletion. Another mechanism was proposed, as increased WBC count indicates leucocytes hypersensitivity to heavy metal and these differences may be due to immunological effects produce antibodies in response to HM-induced stress (22)**.**

Biochemical profile

Total protein levels were recorded decrease with increasing kaolin concentrations in treated groups(Tab.4). Total protein levels of *C.carpio* ranged between 6.60 and 18.19 g/dl in T4 and control-ve respectively, statistical analysis showed a significant differences (P≤0.05) among all treatment groups. Statistical analysis showed that the significant decrease(P≤0.05)in all treatment groups compared with control-ve and significant increase(P≤0.05) in T1 compared with other treatment groups. Also significant decrease (P≤0.05)in T2 compared with T1 and showed significant decrease(P≤0.05) in T3 compared with T2 and T4 compared with T3. Albumin level ranged between 1.31 g/dl in control +ve to 1.53 g/dl in control-ve (Tab.4) .There is no significant different (P>0.05) in all treatment groups. Results of (Tab.4) showed that the level of globulin decreased with increasing concentrations of kaolin in T2,T3 and T4. Globulin level in the serum of experimental fish ranged between 5.15 in T4 and 16.67g/dl in control -ve as showed in Table(4). The globulin concentration showed a significant increase (P≤0.05) in control-ve compared to the other treatment groups. It is also showed a significant increase (P≤0.05) inT1 compared with T2,T3,T4 and control +ve. A significant increase (P≤0.05) of globulin concentration observed in control +ve compared with T2,T3 and T4. A significant increase (P≤0.05) in T2 compared to T3 and T4, and a significant increase (P≤0.05) in T3 compared with T4.

Table 4. Values of biochemical parameters levels(Mean ± SE) of fish exposed to copper sulfate and treated with different concentrations of kaolin during experiment period

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio %
Control -ve	18.19 ±0.19 a	1.53±0.18	16.67 ±0.01 a	0.090 ±0.01 e
Control +ve	12.60 ±0.12 c	1.31±0.05	11.29 ±0.12 c	0.110 ±0.05 d
T1	13.59 ±0.01 b	1.36±0.04	12.23 ±0.01 b	0.110 ±0.02 d
T2	8.40 ±0.06 d	1.34±0.04	7.06 ±0.02 d	0.185 ±0.01 c
T3	7.45 ±0.30 e	1.41±0.01	5.95 ±0.20 e	0.235 ±0.01 b
T4	6.60 ±0.07 f	1.45±0.02	5.15 ±0.05 f	0.280 ±0.02 a
LSD value	0.551 *	0.263NS	0.339 *	0.017 *

Means having different alphabets in columns are significant difference (P≤ 0.05)

The differences in serum albumin concentrations of the fish exposed to copper sulfate showed an increasing trend over time. Albumin has a significant value in laboratory fish because it relates to nutritional status, vascular system integrity, and liver function. Copper sulfate concentrations had a significant impact on total protein, albumin, and globulin. Cu increased the function of copper metalloenzymes which promote body synthesis of body protein. Increase in protein catabolism could be attributed to copper binding with a group of sulfhydryl of protein. In conclusion, this investigation indicated that kaolin has reduced the toxicity effects of copper sulfate in common carp and the best level can be use was 6g/l (T3).

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