EFFECT OF KAOLIN ON BIOCHEMICAL PARAMETERS AGAINST *AEROMONAS HYDROPHILA* IN *CYPRINUS CARPIO*

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ABSTRACT

The present study aimed to evaluate the role of kaolin on hematological and biochemical profiles of common carp, *Cyprinus carpio* infected with *Aeromonas hydrophila*, A total of 180 of common carp were collected 40 g ; 60 fish were used to determine the median lethal dose LD_{50} of *A. hydrophila*, (LD_{50} was 1.57×10^6 CFU/ml) for 15 days. 120 fish were randomly distributed into six treatment groups (10 fish/treatment in duplicate) as follows: C- served as control negative group (without infection and treatment); C+ act as control positive group (infected with *A. hydrophila* but not treatment with Kaolin); T1, T2, T3 and T4 were intraperitoneally (IP) injected with *A, hydrophila* (at dose of 0.1 ml) and then treated with Kaolin at concentrations of 2, 4, 6 and 8 g/L respectively. In hematological indices, the experimental groups of carp (C+, T1, T2, T3 and T4) showed a significant ($P \leq 0.01$) decrease in the number of erythrocytes, Hb content and PCV% compared to C- group. While, WBC numbers showed a significant ($P \leq 0.01$) increase in all experimental carp groups relative to the C- group. As far as biochemical profile are concerned, the experimental groups of carp (C+, T2, T3 and T4) reported a significant ($P \leq 0.05$) increase in total protein compared to C- group. In Globulin, Groups (T2, T3 and T4) showed a significant ($P \leq 0.01$) increase compared to C- group. In conclusion, the treatment of common carp with Kaolin particularly at concentration of 8 g/L (T4) reduced the effect of *A. hydrophila.*

Keywords: albumin, carp, blood, total protein

تأثير الكاولين على الصفات الدمية والكيموحيوية كعوامل مضادة لبكتريا *Aeromonas hydrophila* في اسماك *Cyprinus carpio*

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المستخلص

هدفت الدراسة الحالية إلى تقييم دور الكاولين في المعالجة على بعض الصفات الدمية والكيميوحيوية لأسماك الكارب الشائع *Cyprinus carpio* المصابة ببكتريا *Aeromonas hydrophila* . تم جمع 180نموذجا من اسماك الكارب الشائع بمعدل وزن 40غم، استخدم منها 60 سمكة لتحديد متوسط الجرعة المميتة لـ *A. hydrophila* (1.57×10^6 CFU / ml) لمدة 15 يومًا. بعد ذلك وزعت 120 سمكة عشوائياً الى ست معاملات معالجة (10 أسماك/معاملة مكررة) على النحو التالي: C− مجموعة السيطرة السالبة (بدون اصابة وعلاج). C+ مجموعة السيطرة الموجب (مصابة بـ *A. hydrophila* ولكن لا يتم علاجها بالكاولين) ؛ تم حقن المعاملات T1 و T2 و T3 و T4 داخل تجويف البريتون ببكتريا *A. hydrophila* (بجرعة 0,1 مل) ثم تمت معالجتها بالكاولين بتراكيز 2 و 4 و 6 و 8 غم / لتر على التوالي. اظهرت نتائج الصفات الدمية في المعاملات التجريبية من اسماك الكارب الشائع (C + و T1 و T2 و T3 و T4) انخفاضًا معنويًا ($P \leq 0.01$) في عدد كريات الدم الحمراء ومحتوى الهيموكلوبين ونسبة PCV مقارنة بالمجموعة C−. بينما أظهرت نتائج الكريات الدم البيض زيادة معنوية ($P \leq 0.01$) في جميع المعاملات المعالجة تجريبياً مقارنة بالمجموعة C−. فيما يتعلق بالنتائج الكيموحيوية سجلت المعاملات التجريبية من اسماك الكارب (C + و T2 و T3و T4) زيادة معنوية ($P \leq 0.05$) في البروتين الكلي مقارنة بالمجموعة C − . ، أظهرت نتائج الكلوبيولين في المعاملات (T2 و T3 و T4) زيادة معنوية ($P \leq 0.01$) مقارنة بالمجموعة C−. يمكن ان نستنتج بأن الكاولين قد قلل من تأثير *A. hydrophila* في اسماك الكارب الشائع وأن أفضل مستوى يمكن استخدامه هو 8 غم / لتر . (T4)**

الكلمات المفتاحية: كارب، البومين، دم، البروتين الكلي

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INTRODUCTION

According to FAO (13), the importance of aquaculture as a primary source that can be depended upon to ensure food security in countries, particularly in terms of ensuring animal protein with a high nutritional value as well as conserving and strengthening natural resource stocks and environmental preservation, cannot be overstated (13). *Aeromonas spp*. has the highest death rate for fish infectious illnesses, is extensively spread in the environment, and causes a variety of diseases in both fish and human (26). *Aeromonas spp*. is separated into two phenotypically different categories, according to Fernandez and Figureueras (14), the first group contains psychrophilic (optimum growth at 22–28 °C) or non-motile *Aeromonas* species, whereas the second group includes motile *Aeromonas* species. The clinical signs of the disease were reddish head, anal, and fin bases while the rest of the body was pale. External ulcerative and hemorrhage spots were noticed (20). Kaolinite is known for its capacity to absorb proteins, bacteria, and even viruses onto the surfaces of its crystals, which may then be readily removed (8). They have been used for pharmacological and cosmetic purposes since antiquity (9). Hematological and biochemical indicators can be used to evaluate the health of the organism. One factor that provides a higher oxygen delivery to tissues is a high red blood cell count; in the blood of *C. carpio*, RBC was observed to range from 0.33 to 2.95 $\times 10^6$ cell/mm^3 (32). Even among non-specific people under the same circumstances, the amount and proportion of WBCs in fish blood circulation vary significantly. Reference WBC values in the range of 2.15 to 15.47x10 cell/mm^3 were provided by Hrubec *et al*.(16).One of the most essential indicators of an animal's physiological environment is blood proteins and their concentrations. As a result, proteins play a role in metabolic processes and can be used as a source of energy (19). As a result, exceeding the usual limit of protein content in an animal's blood serum is seen as a sign of excellent health. Food consumption, organism size, sexual maturity, and temperature are all variables that affect total protein levels in fish serum (12, 21, 25). Albumin is a critical serum protein that helps steroid hormones go where they need to go. At the same time, globulins play a crucial role in immune response, such as gamma globulin fractions, which are considered the principal source of all proteins needed for immunological response in blood circulation(17). Kaolin, which is composed of kaolinite, has been widely employed in a wide range of technical applications. Due to their biocompatibility and features like ion exchange and adsorption desorption, microporous crystalline hydrated aluminosilicates, which are similar to zeolites, have several uses (1, 2). Clay-based adsorbents, such as zeolite and clay-based (bentonite, kaolin and montmorillonite), are economical (5, 6, 7). The current is study aimed to assess the effect of kaolin on hematological and biochemical parameters in *C. carpio* infected with *A. hydrophila* .

MATERIALS AND METHODS

Determination the Median Lethal Dose (LD_{50}) of *A.hydrophila:* Using the random sample procedure, fish were separated into six groups, ten fish from each group were intraperitoneally (IP) injected with 0.1 ml volumes of freshly generated (*A. hydrophila*) bacterial suspensions in saline that were 10-4, 10-5, 10-6, 10-7 and 10-8 viable cells/fish. All groups were monitored for 15 days after receiving a 0.1 ml injection of sterile saline as the control group. Standard pathological and microbiological examinations were performed on mortality of fish were also recorded.

Experimental fish

This study was carried out in the College of Veterinary Medicine University of Baghdad/ Fish Disease. A total of 120 healthy *C. carpio* weighing 40 g were purchased from a local carp farm in Al-Mesiab Babylon (Iraq). To get rid of any possible external parasites that may have been present, the fish were dipped in 3 % NaCl until they started to exhibit indications of stress. Then, fish were acclimated for 14 days pre starting the experiment. 120 fish were weighed and divided into six treatments at random (10 fish in duplicates were kept for each treatment) as follows : C- served as control negative group (without infection and treatment); C+ act as control positive group (infected with *A.hydrophila* but not treatment with Kaolin); T1, T2, T3 and T4 were

intraperitoneally (IP) injected with *A.hydrophila* (at dose of 0.1 ml) and then treated with Kaolin at concentration of 2, 4, 6 and 8 g/L respectively.

Hematological parameters

From randomly picked fish (n=4 from each treatment group), blood samples were obtained from the caudal veins. Blood samples were placed in Eppendorf tubes coated with lithium heparin. Blood samples were also collected without heparin, allowed to clot, centrifuged at 7000 g and serum collected and refrigerated until use for biochemical parameters. For determination of RBC and WBC count, Decies fluid was used and the procedure was done according to Thrall *et al*. (31). PCV % was detected in accordance to method described by Achary and Mohanty (3). Hb content was determined using the Drabkins solution kit approach.

Biochemical parameters

Total protein the test was conducted using a Bio (France) colorimetric test kit using the Biuret technique, as reported by Siwicki and Anderson (29) Calculation Total protein = O.D.Sample/O.D.Standard× n ,G/dl: n=5, gm/l: n=50 ,OD= Optical density. According to Dumas and Biggs (10), albumin was determind in serum samples using the BCG technique at a wavelength of 550 nm. Measurement of albumin the approach, which employed a kit from Bio (France), worked on the theory that an albumin reaction with bromocresol green in an acid medium produced a color that could be detected by spectrophotometry. Albumin was subtracted from total protein to get the total globulin fraction (g/dl). Albumin / Globulin ratio calculated by using albumin and globulin data**.**

Statistical analysis

The influence of various factors on research parameters was determined using the SAS (27) software, or Statistical Analysis System. In this study, the least significant difference (LSD) test (ANOVA) was employed to compare means in a meaningful way. Significance was tested at 5% and 1% levels.

RESULTS AND DISCUSSION

Determination the (LD_{50}) of *A.hydrophila*

Results the LD_{50} of *A. hydrophila* at various concentrations were summarized in (Tab.1). LD_{50} precisely equals 1.57×10^6 CFU/ml of fish at a 0.1 ml concentration. When plotted mathematically, at 14 days even. While there was no mortality in the control group or at the concentration of 0.1 ml 1.57×10^4 CFU/fish at 14 days, mortality of 100% was seen at 0.1 ml 1.57×10^8 CFU/ml.

Table 1. Results LD_{50} of *A. hydrophila* in *C. carpio*

Number of fish	Bacterial Dilution CFU/0.10 Ml	Bacterial dilution	Mortality ratio	Died	Survived	Mortality% $\frac{(D)}{(D+S)} \times 100$
6	10^8	10^{-1}	6/6	6	0	100
6	10^7	10^{-2}	5/6	5	1	80
6	10^6	10^{-3}	3/6	3	3	50
6	10^5	10^{-4}	1/6	1	5	20
6	10^4	10^{-5}	0/6	0	6	0
6	control	Saline	0/6	0	6	0

LD_{50}= 0.10 ml 1.57×10^6 CFU/ml

Elwafai *et al*. (11) observed that injected cyprinid loaches intraperitoneally with *A. hydrophila*, they found that the LD_{50} was 2.3 $x10^6$ CFU/ml. Because *A. hydrophila* is often isolated in an aquatic habitat, it is possible to deduce that acute stress factors such as overstocking and poor water quality caused the mass mortality of *C. carpio*. Those findings are so alarming that fish farmers should realize that opportunistic bacteria *A. hydrophila* can contribute high mortality and economic losses to aquaculture. Interestingly, and related to the latter point, the initial bacterial concentration had a significant effect on settling rate of kaolin as demonstrated here using residual turbidity measurements, in contrast to treatments featuring kaolin alone without bacterial cells (5,33).

Hematological parameters

RBCs count showed a significant decrease ($P \leq 0.01$) in all treatment groups (T1, T2, T3 and T4), respectively compared group .No significant different ($P > 0.05$) among T1, T2, T3 and T4. WBCs count in all treatment groups (T1, T2, T3 and T4) recorded a

significant increase (P≤0.01) in comparison to group. (Tab. 2).The highest values were recorded in T1, which showed a marked increase (P≤0.01) in comparison to T2, T3 and T4, respectively. Results of PCV (%) indicated a significant increase (P≤0.01) in all treatment groups: T1 (15.45) %, T2 (16.67) %, T3 (19.71) % and T4 (26.06) % compared to C+ (21.23) %. T1, T2, T3 and T4 were slightly lower than in the control group, but they were still highly significant (P≤0.01). Results of Hb revealed all treatment groups significant increase (P≤0.01) T1 (5.14), T2 (5.55), T3 (6.56) and T4 (8.68) in comparison to C+ (7.07). Interestingly, T1, T2 and T3 were marginally decreased from control group but were not significant (P≤0.01). In addition, there was high significant differences (P≤0.01) observed T4 treated groups.

Table 2. Results of hematological parameters of *C. carpio* infected with *A. hydrophila* and treated with different concentrations of kaolin

Group	RBC×10^6 / mm^3	WBC×10^3 /mm^3	PCV (%)	Hb (g/dl)
C+	1.37 ±0.12 b	29.61 ±3.60 a	21.23 ±0.63 c	7.07 ±0.21 c
C-	1.74 ±0.01 a	11.66 ±0.09 c	32.67 ±0.88 a	10.55 ±0.29 a
T1	1.23 ±0.03 bc	26.67 ±0.30 a	15.45 ±0.18 e	5.14 ±0.06 e
T2	1.04 ±0.02 c	19.58 ±0.18 b	16.67 ±0.82 de	5.55 ±0.27 de
T3	1.27 ±0.06 bc	25.71 ±1.60 ab	19.71 ±0.59 cd	6.56 ±0.19 cd
T4	1.18 ±0.15 bc	24.96 ±3.70 ab	26.06 ±2.70 b	8.68 ±0.90 b
LSD value	0.258 **	6.829 **	3.892 **	1.297 **

Means with small alphabetic letters in the same column are significantly different ** (P≤0.01).

In the present study the lower value of RBCs in T2 and T4 may indicate that RBC are being impacted or destroyed as a result of increased leukocytosis activity in *A. hydrophila*-infected carp (15, 30). WBCs, Hb content and PCV % when compared to the control group. The present findings in line with the study for 15 days demonstrated considerable improvement in RBC count, Hb content (P≤ 0.01), and PCV% (P≤ 0.01) group in comparison to the control group in kaolin treatments, notably in T4 and T3 (6g/L and 8g/L) (23). The rise in WBCs might be linked to a boost in immunological response and antibody synthesis, which aids in the survival and recovery of fish exposed to the toxicant (4, 22, 28).

Biochemical parameters

Total protein levels showed a significantly increased (P≤ 0.05) in all of the experimental group (C+, T1, T2, T3 and T4) compared to control group C- (Table 3), the highest value was recorded in T4 followed by T3. However, there was no significant difference (P> 0.05) between T4 and T1, T2, and T3. Albumin content after the challenge showed a significant difference in all treatments compared to C- group, but there was an arithmetic rise in values. In comparison to the control treatment C+, the post-challenge readings were (1.59, 1.21, 1.33 and 1.28 g/dl, respectively). Globulin levels rose considerably (P ≤0.01) in T2, T3, and T4 (1.72, 1.62 and 1.72 g/dl, respectively) in comparison to control treatment (1.33 g/dl) (Table 4.5). The best ratio was observed in T4, but there was no significant difference (P>0.05) between T3, T2 and T1. Albumin, globulin contents and A/G ratio showed a significant difference in all treatments post challenge.

Table 3. Results of biochemical parameters of *C. carpio* infected with *A. hydrophila* and treated with different concentrations of kaolin

Group	Mean ± SE			
	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G Ratio
C+	2.28 ±0.01 a	1.68 ±0.06 a	1.36 ±0.04 bc	1.24 ±0.08 a
C-	1.80 ±0.13 c	1.37 ±0.08 b	1.33 ±0.15 c	1.08 ±0.21 ab
T1	2.02 ±0.11 bc	1.59 ±0.03 a	1.26 ±0.05 c	1.26 ±0.06 a
T2	2.07 ±0.08 ab	1.21 ±0.11 b	1.72 ±0.10 a	0.716 ±0.11 c
T3	2.15 ±0.08 ab	1.33 ±0.02 b	1.62 ±0.04 ab	0.824 ±0.02 bc
T4	2.20 ±0.03 ab	1.28 ±0.06 b	1.72 ±0.06 a	0.753 ±0.06 bc
LSD value	0.267 *	0.207 **	0.272 **	0.331 **

Means with small alphabetic letters in the same column are significantly different *(P≤0.05), ** (P≤0.01).

These findings are consistent with Kumar *et al*. (18). The drop in total serum protein and albumin in infected fish is can due to blood vessel injury, which causes a large quantity of blood components to be lost, resulting in hypoproteinaemia. Results of albumin level showed significant increase (P≤0.01) in all treatment groups T1, T2, T3 and T4 compared to infected group C+. This means that the fish regain the health status and started recover. Globulin levels showed significantly (P≤0.05) increased in all experimental groups (T1, T2, T3 and T4). This rise in treated groups may be attributed to an increase in immune response to kaolin. These results are consistent with those of Yildirim-Aksoy *et al*. (33). Globulin increases may be attributed to immunological activity, and their level increase as a defensive reaction in fish exposed to toxicity. At the end of the experiment, the A/G ratio resulted in a substantial rise (P ≤0.01) in T1, T2, T3, and T4 of *C. carpio* when compared to C- and a significant difference (P ≤0.01) when compared to C+. This might be a marker of inflammation (24). In conclusion, the treatment of common carp with Kaolin particularly at concentration of 8g/L reduced the infected of *A. hydrophila*.

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