ROLE OF KAOLIN ON HEMTOLOGICAL, BIOCHEMICAL AND SURVIVAL RATE OF *CYPRINUS CARPIO* CHALLENGED WITH *PESUYDOMONAS AERUGINOSA*

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ABSTRACT

In present study, we assessed the role of kaolin [($Al_2Si_2O_5(OH)_4$), an inert clay], for treatment of common carp, *Cyprinus carpio* experimentally infected with *Pesudomonas aeruginosa.* Fish were experimentally challenged with *P. aeruginosa* ($LD_{50=}$ $2\pm0.2\times10^9$ CFU/ml), in untreated water or water treated with addition of kaolin at levels of 4, 6 and 8g/l (G1, G2 and G3 respectively). Over the 5-day course of kaolin treatment led to significantly ($P \leq 0.05$) improved of survival (100%) in treated groups (G2 and G3) as compared to untreated fish (infected group; 75% survival). Considerable changes were observed in blood parameters, leucocyte count recorded significant increase ($P \leq 0.05$) in G2 and G3 relative to C- and C+ groups. Erythrocyte count, Hb content and HT(%) reported significant decrease ($P \leq 0.05$) in all treated groups (G1, G2 and G3) related to C- group. Globulin level registered significantly increased ($P \leq 0.05$) in G2 and G3 than C+ group. The current study strongly recommends that treatment of common carp infected with kaolin at 6 and 8g/l improves survival, hematological and biochemical profile, against *P. aeruginosa*. Future complete molecular studies are required before application of kaolin as a treatment in aquaculture.

Keywords: Fish, Blood, Total protein, Mortality

دور الكاولين في الصفات الدمية والكيموحيوية ومعدل البقاء في اسماك الكارب الشائع *Cyprinus carpio* المصابة تجريبيا ببكتريا *Pesuydomonas aeruginosa*

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المستخلص

هدفت الدراسة لتقييم دور الكاولين [($Al_2Si_2O_5$ (OH) $_4$)، وهونوع من الطين]، في معالجة اسماك الكارب الشائع *Cyprinus carpio* المصابة تجريبيا ببكتريا *Pesudomonas aeruginosa*. لهذا الغرض, تم اصابة الاسماك تجريبياً ($LD_{50}=2\pm0.2\times10^9$ CFU/ml)في مياه غير معالجة أو الماء المعالج بإضافة الكاولين بنسب 4 و 6 و 8 غم/لتر (G1 و G2 و G3 على التوالي). على مدى 5 أيام من العلاج بالكاولين اظهرت النتائج فروقات معنوية ($P \leq 0.05$) في معدل البقاء (100٪) في المعاملتين المعالجة (G2 و G3) مقارنة بالأسماك غير المعالجة (معاملة السيطرة الموجبة) التي بلغت فيها نسبة البقاء 75٪، كما لوحظت تغيرات كبيرة في المعايير الدمية، اذ سجلت اعداد كريات الدم البيضاء زيادة معنوية ($P \leq 0.05$) في G2 و G3 مقارنة بمعاملتي السيطرة السالبة والموجبة، كما سجلت اعداد كريات الدم الحمراء ومحتوى الهيموكلوبين وحجم كريات الدم المضغوطة انخفاضًا معنويًا ($P \leq 0.05$) في المعاملات المعالجة (G1 و G2 و G3) مقارنة بمستوى الهيموكلوبين في معاملة السيطرة السالبة. سجل معدل الكلوبيولين زيادة معنوية في المعاملات المعالجة (G2 و G3) مقارنة بمعاملة السيطرة الموجبة. توصي الدراسة الحالية بشدة أن علاج الكارب الشائع بالكاولين عند 6 و 8 غم/ لتر يحسن من معدل البقاء والخصائص الدمية والكيموحيوية، ضد بكتريا *P. aeruginosa*. يتطلب اجراء دراسات جزيئية كاملة في المستقبل قبل تطبيق الكاولين كعلاج في تربية الأحياء المائية.

الكلمات المفتاحية: أسماك، الدم، البروتين الكلي، هلاكات

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INTRODUCTION

Several bacterial diseases continue to pandemic the Iraqi farmed fish industries which are responsible for tremendous economic losses. Generally, Pseudomonads are one of the most serious pathogen in the aquaculture, resulting for heavy losses in the farmed fishes (33). *Pesudomonas aeruginosa* is considered as a causative agent of nosocomial infections induce hemorrhagic septicemia and ulcerative syndrome in various fish species, causing high mortality in fish producing industry worldwide (5,33, 34). In general these bacteria found as a part of the normal fish microbiota, but the infection with *P. aeruginosa* is associated with changes in normal environmental conditions (low environmental quality). Infections with *P. spp.* have been reported in freshwater fish can causes a long-duration chronic disease, predominantly affecting immune system defense (3). "Besides, *Pseudomonas* can be a problematic for human consumers also. They appear in processes of seafood spoilage (16, 17) and in ready-to-eat foodstuffs (30).In some conditions they can become human pathogens and cause infection. In general, application of antibiotic to control fish pathogens has been limited or eliminated in several countries due to concern associated to cross-resistance to antimicrobial agents that are used in human medication" (7). Due to this limitation, there is a frantic requirement for new therapies and preventatives that can help manufacturers decrease overpriced disease related harms. Recently, natural clay have been testified as immuno-stimulant, enhance growth, conversing disease resistance by improving immunity in both fishes and shellfish (10, 11, 18, 20, 22, 23, 29, 35). Kaolin ($Al_2Si_2O_5(OH)_4$), is a clay which has a long history of medicinal use, particularly in the context of gastrointestinal disease. Treatment with natural clay as an immune- stimulant and therapy is estimated nontoxic in both the fish health and ecosystem in aquaculture (2).Thus, this study was undertaken to assess the role of kaolin on hematological, biochemical tests and survival of common carp experimentally infected with *P. aeruginosa.*

MATERIALS AND METHODS

Isolation and identification of *P. aeruginosa*

Briefly, specimens were collected from internal organs of fish were directly streaked onto tryptic soy agar (TSA) plate and left incubated for 24 h. at 27 °C under aerobic condition. The synthesis of yellowish- green fluorescent pigment is usually related with *Pseudomonads spp*. (20). All suspected colonies were harvested and purified for biochemical and phenotypic characteristics. All isolates were recognized morphologically by Gram's stain and by using different biochemical sets according to manufacture instructions; urease, catalase, indole, oxidase, Voges Proskauer, citrate utilization, mannitol fermentation, H_2S production, and gelatin hydrolysis, in addition to for their motility using "hanging drop method"(24).Further identification of the isolates was performed using "Analytical Profile Index (API) test kits 20E and 20NE (Biomerieux)", according to the manufacturer instructions.

Kaolin clay

Kaolin "($Al_2 Si_2O_5(OH)_4$) was obtained from Ministry of Industry and Minerals, State Company For Mining Industries, Iraq in form of 10 Kg white powder package".

Experimental fish design

A total of 80 healthy *C. carpio* (60.8 ± 4.3g; length 20.3±0. cm) were purchased from a local fish farm from Babylon, Iraq. "Fish were immediately inspected their health status upon entrance. Then, fish were dipped in treated solution with 2% sodium chloride a period of 5 min. and acclimated for 12 days in 80 L (50×60×100cm) aerated glass tanks previous to experiment. After acclimation, the fish were divided into five groups of 16 fish/treated group in duplicate (16×5 =80 fish) as: control negative (C-) group uninfected and without kaolin ; (C+): fish were infected with *P. aeruginosa* and without kaolin treatment. Group 1, 2 and 3 fish were infected with *P. aeruginosa* and treated with kaolin at levels of 4, 6, 8 g/l for 5 successive days respectively. These rates of kaolin were selected based on study of Eissa et al. (13) with some changes". Fish were fed on standard commercial diet every day at rate of 2% body mass. Tanks were cleaned daily and water exchanged partially. Physical and chemical parameters

were registered daily as: (dissolved oxygen 6.7±1.40 mg/l; temperature 22.03±1.40 °C and pH 7.4±0.6). Fish were monitored during the experimental period, dead fish were removed and survival was reported. Over the 5-day course of kaolin treatment, three fish from each tank were selected randomly; blood samples were collected from caudal vein for determination of blood and biochemical tests.

Hematological tests

At the end of the experiment, three fish from each tank were selected randomly. Blood samples were collected from caudal vessel, blood parameters including red blood cells (RBC) and white blood cells (WBC) count were counted by Neubaur's upgraded haematocytometer (Marienfeld, Superior, Germany) using Deices as a diluting fluid (26,27). Hemoglobin content and hematocrit percentage were detected according to procedure of Al-Rudainy et al. (4).

Biochemical tests

The serum total protein content was measured by "Biuret colourimetric reactions" (6) while the serum globulin and albumin contents were determined by bromocresol green colourimetric reaction (5, 28). Determination of sugar concentration in serum was done using a commercial kit "(Glu L 1000, PLIVA-Lachema, Czech Republic)".

Statistical analysis

Statistical analysis was performed using "SPSS Inc., Chicago, IL, version 20". Data were analyzed as "average ± SE". "Contrast among groups was conducted using a one-way analysis of variance (ANOVA). A probability equal or less than 0.05 ($P \leq 0.05$) was measured significantly different".

Hematological tests

Considerable changes were observed in blood parameters as shown in Table 1. "Erythrocyte count, Hb content and HT(%) reported significant decreased ($P \leq 0.05$) in treated groups (G1, G2 and G3) compared to C- group. On the other hand, leucocyte count recorded significant increase ($P \leq 0.05$) in G2 and G3 relative to C- and C+ groups. Hematological indices offer a valuable data in the assessment of fish health status and stress response. The increase in total leucocyte count could be owing to an early sign of innate defense and adaptive immune response (10) which is first stage of defense mechanism". The WBC counts increased in infected and treated fish (kaolin treated with 4, 6 and 8g/l). Similar results have been reported with in *Oreochromis niloticus* treated with kaolin (12) and in *Ctenopharyngodon idellus* fed with Kaolin incorporated diet and challenged with *Aeromonas hydrophila* (19).

Biochemical Tests

Total protein showed significantly increased groups. Globulin level registered significantly increased in G2 and G3 than C+ group. "Albumin level reported significantly increased in G2 and G3 than C- group. As well as, Sugar level showed significantly increased in G2 and G3 than C- and G+ groups (Table 2). In the present study the total protein level was significantly increased in fish treated with kaolin 6 and 8g/l. The serum albumin values were also increased in infected and fish treated with 6 and 8g/l. These results favorably relate with that of *Ctenopharyngodon idellus* fed with Kaolin incorporated diet and challenged with *A.hydrophila* (22) when the albumin content decreases the fluids could leakage into tissues resulting localized oedema decreasing the transport of nutrient to tissues (21)". which is a reliable prognostic indicator for increased risk of morbidity and mortality (11) The increasing of serum globulin level in fish treated with 8g/l kaolin perhaps owing to the B- lymphocytes stimulation, differentiation and proliferation by IL-6 and TNF-α (31) that is often occupied in chronic stages by different viral, parasites and bacterial infections (1).

Survival rate

The highest survival rate (100%) was seen in kaolin treated groups (G2 and G3) followed by G1 group the survival was 93%. While, the lowest survival rate was observed in C+ group (75%) (Table 3).Concerning the pathogenicity experimental, common carp challenged with *P. aeruginosa* showed high mortality rate in infected group (C+) in direct proportion to the "encoded virulence genes". Our findings asserted that treatment with kaolin post to the challenge of *C. carpio* protected the fish against *P. aeruginosa* as the survival improved to 93 and 100% in kaolin-treated challenged group, while reached to 75% in untreated challenged group. This result is in complete

agreement with (8) and Eissa et al. (14) whom reported survival improvement in infected channel catfish by the reduction of bacterial attachment to target tissues of columnaris disease. They stated that Kaolin adsorbs the bacterium and binds to it, hence, inhibiting it from attaching to the fish and causing the disease. Due to of its adsorbent ability, kaolin was applied in hatchery processes in aquaculture to reduce egg adhesion (25) as well as added to fish diets to prevent enteric diseases via its capability to absorb enterotoxins and major mycotoxins (9,12,33).

CONCLUSIONS

P. aeruginosa is one of the major persistent evolving pathogens commonly isolated from *C. carpio.* The present study indicated that Kaolin not only provides producers an alternative therapy to antibiotics in fighting *P. aeruginosa*, but it also offers an uncostly treatment for a pricey fish diseases since "plentiful clay" is available in Iraq.

Table 1. Blood indices in common carp challenged with *P. aeruginosa* and treated with different levels of kaolin

Treated groups	RBC count $\times10^6$/µl	WBC count $\times10^3$/µl	Hb content g/dl	Ht %
Cont.(-)	1.55±0.10^a	15.30±3.62^c	8.60±1.35^a	25.81±2.33^a
Cont.(+)	1.39±0.05^b	18.12±3.11^b	7.40±0.45^b	22.20±0.78^b
G1	1.37±0.08^b	19.50±2.41^b	6.60±0.62^b	19.90±1.08^b
G2	1.46±0.25^c	22.40±2.40^a	5.60±0.15^{bc}	16.80±0.26^{bc}
G3	1.42±0.30^{bc}	22.90±2.05^a	6.80±0.14^{bd}	20.60±0.25^{bc}
LSD	0.09*	3.02*	1.12*	3.48*

Data with small alphabetic letters are indicated significant differences at P≤0.0.5

Table 2. Biochemical assays in common carp infected with *P. aeruginosa* and treated with different levels of kaolin

Treated groups	Total protein g/dl	Albumin g/dl	Globulin g/dl	Sugar g/dl
Cont.(-)	3.50±0.80^c	1.28±0.31^b	2.21±0.21^b	74.91±16.66^c
Cont.(+)	2.75±0.50^d	1.64±0.32^a	1.10±0.02^c	113.66±33.66^b
G1	2.83±0.60^d	0.93±0.04^c	1.90±0.03^b	97.07±12.32^b
G2	4.64±0.50^b	1.65±0.01^a	2.71±0.09^b	152.66±24.22^a
G3	6.33±0.62^a	1.53±0.05^a	4.79±0.02^a	155.89±14.03^a
LSD	0.65*	0.31*	0.71*	20.54*

Data with small alphabetic letters are indicated significant differences at P≤0.0.5

Table 3. Survival rate in common carp challenged with *P. aeruginosa* and treated with different levels of kaolin

Treated groups	No. of fish	Challenge with *P. aeruginosa*	Kaolin treated	Dead fish	Survival rate %
Cont.(-)	16	Non	Non	0	100
Cont.(+)	16	Challenged	Non	4	75
G1	16	Challenged	Treated	1	93
G2	16	Challenged	Treated	0	100
G3	16	Challenged	Treated	0	100

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