EFFECT OF KAOLIN ON HISTOPATHOLOGICAL ALTERATIONS IN *CYPRINUS CARPIO* CHALLENGED WITH *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

This study aimed to assess the role of kaolin in reducing the adverse effect of *Pseudomonas aeruginosa*in common carp, *Cyprinus carpio*. Specifically, the histopathological alterations in kidney, spleen and liver were investigated. Firstly, *P. aeruginosa* was isolated from infected fish. A total of 100 fish (40.8 ± 2 g)were divided into five groups in duplicate (10 fish in each replicate) as:control healthy (C-) group uninfected and without kaolin treatment; (C+): fish were infected with *P. aeruginosa* and without kaolin treatment. Group T1, T2 and T3 fish were infected experimentally with *P. aeruginosa* ($LD_{50=}$ 2±0.2×10^9 CFU ml^{-1}), andtreated with kaolin at concentrations of 4, 6, 8 g/L for 7 successive days respectively.Kidney sections in positive control showedhydrobic degeneration, reduction of hemopotic tissue, and nuclear pyknosis. Spleen sectionsexhibited melanomacrophage aggregation and congestion in red bulb of the positive control. While, liver sections revealed necrosis of hepatic cells and cellular vacuolation. All of these alterations were lesser of extent in kidney, spleen and liver in kaolin treated groups. Overall, this study provides insights into the potential use of kaolin as a treatment for against *P. aeruginosa* in common carp and may contribute to the understanding of the mechanisms underlying histopathological alterations in response to bacterial infections. As well as, histopathological alterations provides detailed information and a powerful tool about the cellular and subcellular structure of tissues and organs.

Keywords: common carp- hemorrhagic septicemia-kidney-spleen

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تأثير العلاج بالكاولين على التغيرات النسجية المرضية في اسماك الكارب الشائع *Cyprinus carpio* المصابة تجريبيا ببكتريا *Pesuydomonas aeruginosa*

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المستخلص

هدفت الدراسة تقييم دور الكاولين في تقليل التأثير لبكتريا*Pseudomonasaeruginosa* في اسماك الكارب الشائع*Cyprinus carpio*. تم فحص التغيرات النسجية المرضية في الكلى والكبد والطحال. أولاً ، تم عزل *P. aeruginosa* من الأسماك المصابة. تم تقسيم 100 سمكة إلى خمس معاملاتبمكررين (10 سمكة في كل مكرر) على النحو التالي: معاملة السيطرةالسليمة (C−) غير المصابة وبدون علاجبالكاولين. (C+): أصيبت الأسماك ببكتيريا *P. aeruginosa* وبدون علاج الكاولين. أصيبت المعاملاتT1 و T2 و T3 تجريبياً بالبكتيريا ا (LD_{50} = 2 ± 0.2 × 10^9 CFU ml^{-1}) وعولجت بالكاولين بتراكيز 4 و6 و 8 غم/ لتر لمدة 7 أيام متتالية. أظهرت المقاطع النسجيةللكلى في +C تنكسًا مائيًا ، وتقلصًا في الأنسجة الدموية ، وتغلظ في الانوية. أظهر نسيج الطحال تراكم الميلانوما واحتقان في اللبالاحمرلمعاملة+C. ، بينما أظهرت مقاطع الكبد تنخر الخلايا الكبدية والفجوات الخلوية.كانت كل هذه التغييرات أقل تاثيرا في الكلى والكبد والطحال في المعاملات المعالجة بالكاولين. بشكل عام ، تشير هذه الدراسة حولامكانيةاستخدام الكاولين كعلاج بكتيري في اسماك الكارب الشائع وقد تساهم في فهم الآليات الكامنة وراء التغيرات النسجية المرضية استجابة للعدوى البكتيرية. علاوةعلى ذلك، توفر التغيرات النسجية المرضية معلومات مفصلة وأداة قوية حول البنية الخلوية وشبه الخلوية للأنسجة والأعضاء.

الكلمات المفتاحية: الكارب الشائع، الانتان الدموي، الكلية، الطحال

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INTRODUCTION

Pseudomonads as one of the most serious pathogens responsible for significant economic losses. Particularly, *Pseudomonas aeruginosa* is highlighted as a causative agent of "nosocomial infections, hemorrhagic septicemia, and ulcerative syndrome in numerous fish species, resulting in high mortality rates and significant negative impacts on the global fish production industry". Pseudomonas can also be problematic for human consumers (20). Additionally, Pseudomonas have been identified in seafood spoilage and ready-to-eat foodstuffs, which can pose a risk to human health if consumed. The use of antibiotics to control fish pathogens has become a contentious issue in recent years, as there is increasing concern over the potential for cross-resistance to antimicrobial agents that are also used in human medicine. This means that the use of antibiotics in aquaculture could contribute to the development of antibiotic-resistant bacteria that could potentially increasetoxicity, residues causing public health and environmental problems (15) lately, new clay such as kaolin has been reported to have various beneficial effects on the growth and health of fish and shellfish (3, 6,18, 19). Several studies have demonstrated that the inclusion of kaolin in fish and shellfish diets can enhance growth performance, improve feed conversion efficiency, and boost immune function. Additionally, kaolin has been shown to have a protective effect against various pathogens, such as bacteria and viruses, by increasing the animal's natural defenses (2, 8, 9, 25). Kaolin, a natural clay substance with many applications in the treatment of bacterial diseases. Kaolin is a hydrated aluminum silicate clay material synthesized from the mineral kaolinite, which is found in the crust of the earth (15, 16). Primary kaolinite deposits are produced through low-temperature hydrothermal alteration of aluminum-rich silicates found in rocks such as rhyolites, granites, and quartz diorites. Secondary kaolinite deposits are found in non-marine habitats where its parent minerals have been transported under non-alkaline circumstances (3, 21). There is no evidence of side effects on the host health; neither there has been any adverse effect in hematological and biochemical parameters. It has a protective effect against entero-toxigenic strains like *Escherichia coli* (23). Based on above information, therefore the aim of this study to assess the role of kaolin on histopathological alterations in common carp, *Cyprinus carpio* experimentally infected with *P. aeruginosa.*

MATERIALS AND METHODS

Pathogen Isolation and identification *Pseudomonas aeruginosa*was isolated from the kidney and liver of an infected carp fish displaying several clinical signs. Bacterial isolation was done according to method of Suhail (7). The bacteria were grown and sub-cultured on tryptic soy agar plates, refined, and re-suspended in either 0.85% NaCl or PBS. The concentration of the bacterial cell suspension was determined using "a Neubauerhaemocytometer", which yielded a concentration of 0.2×10^9 CFU ml^{-1}. Various biochemical tests to characterize this bacterium were performed using biochemical characterization of the bacterial cells using microbial biochemical identification tubes according to the manufacturer's instructions. In addition to, "Analytical Profile Index (API) test kits 20E and 20NE (Biomerieux)" were used to identify the bacterial species.

Kaolin material: The kaolin used in this work attained from the "State Company Form Ministry of Industry and Minerals" in Iraq in the form of a 10 Kg as a white powder package. This type of kaolin is likely a high-quality, pure form of the mineral, which makes it suitable for various industrial and scientific applications.

Experimental set up: A total of 100 healthy common carp weighing approximately 40.8 ± 2 g and length 18±1.8 cm were purchased from a local fish farm (Babylon/Iraq). Upon arrival, the fish were examined to ensure their health status. Following the health check, the fish were dip-treated with a 2% NaCl solution, which is a common disinfectant used in aquaculture to reduce the risk of bacterial and fungal infections. After the dip treatment, the fish were acclimated for 12 days in 80 L aerated fiber tanks before the start of the experiment. During the acclimation period, the fish were monitored closely for any signs of stress or illness and were fed a suitable diet to ensure optimal health and growth. Following the acclmation period, the fish were divided into five groups of 10 fish in each replicate (5 groups ×10 fish × 2 replicates= 100 fish) as: control healthy (C-) group uninfected and without kaolin treatment ; (C+): fish were infected with *P. aeruginosa* and without kaolin treatment. Group 1 (T1), 2 (T2) and 3 (T3) fish were infected experimentally intraperitoneally with *P. aeruginosa* ($LD_{50=}$ 2±0.2×10^9 CFU ml^{-1}), andtreated with kaolin at concentrations of 4, 6, 8 g/L for 7 successive days respectively. These rates of kaolin were selected based on study of Al-Rudainy et al. (3). During the experiment, the fish were fed a standard commercial diet daily, at a rate of 2% of their body mass. This ensured that the fish received adequate nutrition to support their health. The tanks were cleaned daily to maintain water quality. Partial water exchanges were also performed regularly to further improve water quality. The physical and chemical parameters of the water were monitored daily to ensure that they remained within suitable ranges for the fish. Dissolved oxygen levels were measured at 6.8±1.30 mg/L, the temperature was maintained at 23.02±1.40 °C, and the pH was kept at 7.6±0.3. At the end of the experiment Fish were dissected for histopathological study.

Histopathological examination: For histopathology, the procedure was done based on the protocols developed by Mustafa and Ashor (6). The samples were first dissected and then placed in a neutral buffered formaldehyde solution (10%) to preserve their internal structure. The samples were then subjected to a series of treatments including rinsing, dehydration (using graded ethanol concentrations), embedding in paraffin wax with a melting point of 54-56°C, and sectioning using a rotary microtome. The resulting sections (5-8 µm thickness) were stained with "hematoxylin and eosin (H&E) for histopathological examination under a light microscope". This method allows for detailed analysis of the internal structure of the fish organs, providing insights into their anatomy and pathology.

RESULTS AND DISCUSSION

Kidney sections of *C. carpio* of control group displayed normal structure of the renal tubules and hemopoietic tissue. Positive control demonstrated reduction of hemopotic tissue, detached of epithelial cells from basal lamina with hydrobic degeneration and nuclear piknosis of renal tubules. While, T1 showed hydrobic degeneration with mild melanomacrophage aggregation. Wearers, T2 and T3 showed melanomacrophage aggregation (Figure1 A-F).Spleen sections revealed normal structure in C- while the C+ showed congestion with melanomacrophage aggregation. Kaolin treated groups showed melanomacrophage aggregation (Figure 2 A-D).Liver sections revealed normal appearance of the control group. Positive control exhibited necrosis of hepatic cells, nuclear piknosis and dilation of sinusoids. All of these changes were minor in extent in Kaolin treated groups (Figure 3 A-F). One of the key benefits of histopathology is its ability to provide detailed information about the cellular and subcellular structure of tissues and organs. This information can be used to diagnose diseases and to monitor the progression of disease over time. It can also be used to study the effects of drugs or other treatments on tissues and organs (6, 14). Another benefit of histopathology is that it can be used to investigate the underlying causes of disease, such as genetic mutations or environmental factors (17, 18). This information can help to develop new treatments and preventive measures to reduce the incidence and impact of diseases. In the present study, kaolin treatment markedly reduced the lesions in tissues of common carp. kaolin has several benefits in comparison to the use of antibiotics or other chemical agents. One of the most significant advantages is that kaolin does not contribute to the development of antibiotic resistance in bacteria, which is a growing concern in healthcare and agriculture. Antibiotic resistance occurs when bacteria develop the ability to survive exposure to antibiotics, making it more difficult to treat infections.Additionally, the use of kaolin does not introduce harmful chemical residues into the environment or consumer products, which can have negative impacts on human and environmental health (23). One of the mainhistopathological changes in the current study involvedmelanomacrophage aggregation specially in the spleen. The reason of the hemosiderosis was reported to be associated with β-hemolysin that result hemolysis inside the fish body followed by deposition of hemosiderin (13). Liver tissue showed necrotic hepatocyte in C+ group. These changes of tissue destruction was documented in the walking catfish, *Claris batrachus*by Angka(4), Nile tilapia, *Oreochromisniloticus*(1), infected experimentally with *Aeromonas hydrophila*. The reason of the necrosis of liver and kidney was described to be associated with the release of toxins and extracellular products such as hemolysin, protease, elastase produced by *Pseudomonas aeruginosa*(5), which could result to rapid death in severe cases (5).It is possible that the histopathological alteration observed in the spleen are could be due to the spleen being the primary initial target organ for the invading pathogen or due to the important role that the spleen plays in monitoring the blood stream against invading pathogens. The spleen is also a vital immune organ that plays a crucial role in filtering the blood and responding to foreign antigens. In some infections, the spleen can become enlarged and dysfunctional, leading to a range of pathological changes (22, 24). The specific reasons for the histopathological changes observed in the spleen would depend on the nature of the pathogen and the specific immune response elicited by the host. Different pathogens can elicit different immune responses and cause different pathological changes in the spleen. Therefore, it is essential to identify the specific pathogen responsible for the infection and characterize the immune response elicited by the host to understand the histopathological changes observed in the spleen (10, 11, 12, 19). “In some infections, such as malaria, the spleen can become enlarged and dysfunctional, which can lead to a range of pathological changes. However, the specific reasons for the histopathological changes observed in the spleen would depend on the nature of the pathogen and the specific immune response elicited by the host” (20).

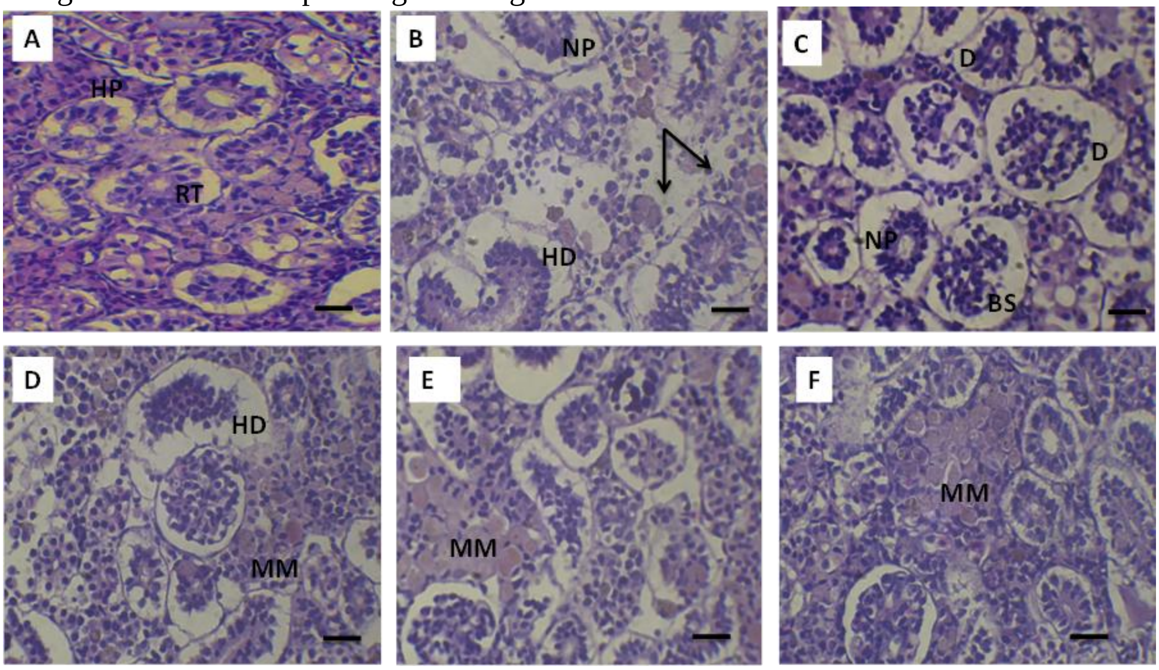

Figure 1. Photomicrographs of the kidney tissue of *C. carpio* treated with different concentrations of kaolin and experimentally infected with *Pseudomonas aeruginosa*A: control showing normal structure of the renal tubules (RT) of kidney and hemopoietic tissue (HP) B &C: positive control (C+) demonstrating reduction of hemopotic tissue (arrow), detached of epithelial cells from basal lamina (D) with hydrobic degeneration (HD) and nuclear piknosis (NP) of renal tubules D: T1 showing hydrobic degeneration (HD) with mild melanomacrophage aggregation (MM). E&F : T2 & T3 showing hemosiderosis together with melanomacrophage aggregation (MM). H & E stain. Thickness = 5 mm. Scalebars= 50 mm

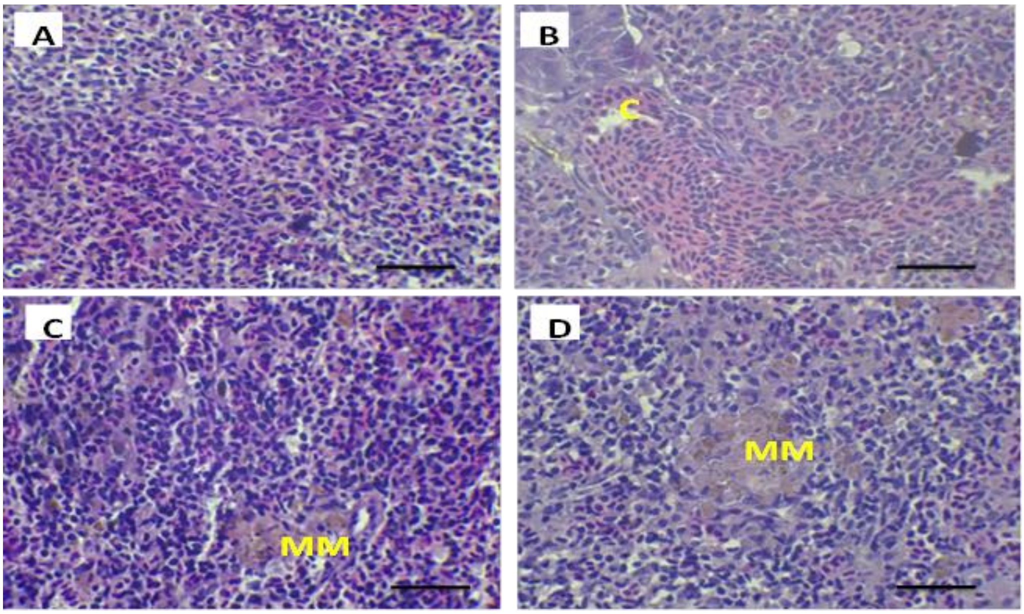

Figure 2. Photomicrographs of the spleen tissue of *C. carpio* treated with different concentrations of kaolin and experimentally infected with *Pseudomonas aeruginosa*A: control showing normal structure) B &C: positive control (C+) demonstrating congestion(C) and melanomacrophage aggregation (MM) ; D: represented T1, T2 and T3 showing melanomacrophage aggregation (MM). Thickness = 5 mm. Scalebars= 50 mm

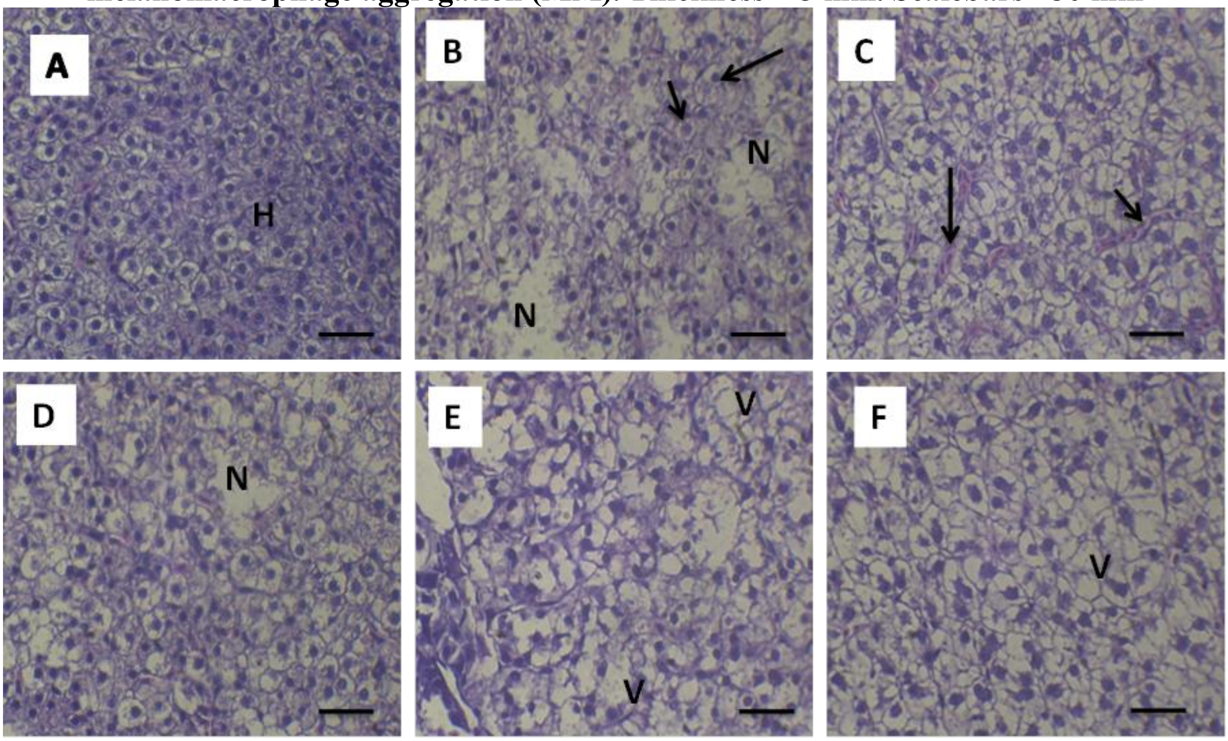

Figure 3.Photomicrographs of the hepatic tissue of *C. carpio* treated with different concentrations of kaolin and experimentally infected with *Pseudomonas aeruginosa*A: control showing normal structure of the hepatic tissue (H) of liver B &C: positive control (C+) demonstrating necrosis of hepatic cells (N), nuclear piknosis (black arrow) and dilation of sinusoids (arrows) D: T1 showing some areas of necrosis (N). E&F : T2 & T3 showing cellular vacuolation (V). H & E stain. Thickness = 4 mm. Scalebars= 50 mm.

CONCLUSIONS

Overall, this study provides insights into the potential use of kaolin as a treatment for against *P. aeruginosa* in common carp and may contribute to the understanding of the mechanisms underlying histopathological alterations in response to bacterial infections. As well as, histopathological alterations provides detailed information and a powerful tool about the cellular and subcellular structure of tissues and organs. This information can be used to diagnose diseases and to monitor the progression of disease over time. It can also be used to study the effects of drugs or other treatments on organs and tissues.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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