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Interaction of Zinc Nanoparticles with Malathion on Nile Tilapia

Nihal Kahwagy[1], Gehad El-Sayed[1], Mohamed El-Adl[1] and Nevien Abdelkhalek[2]

[1]*Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine Mansoura University, Egypt.*

[2]*Department of Aquatic Medicine, Faculty of Veterinary Medicine, Mansoura University, Egypt.*

Abstract

AQUACULTURE has emerged as a crucial approach to sustainably meet the growing global demand for protein. Nile tilapia (*Oreochromis niloticus*), a prominent freshwater species, is important in aquaculture and culinary applications. This research examined the interaction of zinc oxide nanoparticles and malathion pesticides on Nile tilapia. Nile tilapia received 2 mg/L Zinc oxide nanoparticles and/or 3 mg/L Malathion. After thirty days, serum samples were collected for the determination of hepatic damage markers, hepatic and branchial antioxidant status, and gene expression analysis for immune genes. A significant decrease in the ALT/AST ratio was detected in all treated groups. Zinc oxide nanoparticles improved antioxidant status and decreased lipid peroxidation levels in the malathion-intoxicated group, significantly reducing proinflammatory cytokines. This study highlighted the potential and specificity of zinc oxide nanoparticles in alleviating the toxicity of malathion in Nile tilapia which gave insights into ameliorating the toxicity of pesticides with physiological metal nanoparticles in aquaculture.

Keywords: Malathion, Nanoparticles, Tilapia, Zinc oxide.

Introduction

Aquaculture has emerged as a crucial approach to sustainably meet the growing global demand for protein. As the need for aquatic food sources increases, it becomes essential to adopt efficient and productive aquaculture practices. Recently, zinc nanoparticles (ZnNPs) have garnered attention for their role in enhancing aquaculture outcomes. ZnNPs bolster fish health by enhancing immune responses and reducing disease prevalence, making them a valuable addition to fish farming strategies. Their inclusion in feed and water treatments enhances growth rates, optimizes feed efficiency, and minimizes environmental impact, reinforcing their significance in advancing aquaculture productivity [1].

Nile tilapia (*Oreochromis niloticus*), a widely cultivated freshwater fish, is highly valued in both aquaculture and cuisine [2]. Zinc oxide nanoparticles (ZnNPs), with a diameter less than 100 nm, possess unique properties and are utilized across various fields [3]. The precipitation method is a reliable and controllable approach for synthesizing ZnNPs, employing capping agents to prevent agglomeration. Key factors such as temperature and calcination significantly influence nanoparticle formation [4].

Nayak et al. successfully synthesized ZnNPs through direct precipitation using zinc sulfate, adipic acid, sodium hydroxide, and distilled water. The process involved stirring the reactants to produce a precipitate, followed by filtration and washing with distilled water and acetone to remove residual adipic acid. The final product was dried under ambient conditions for several hours [5].

ZnNPs provided an innovative solution for enhancing aquaculture efficiency. Their small size and large surface area improve zinc bioavailability, allowing superior absorption compared to traditional zinc sources [6]. This enhanced absorption promotes better growth rates, improved feed conversion efficiency, and overall fish health [7]. Additionally, ZnNPs exhibit potent antimicrobial properties, aiding in pathogen control, reducing the necessity for antibiotics, and fostering a healthier aquaculture environment. By mitigating oxidative stress, ZnNPs promote cellular health and immune function, making them a promising resource for sustainable and eco-friendly fish farming [8].

*Corresponding authors: Mohammed El-Adl, E-mail: mohamedel_adl@mans.edu.eg Tel.: +201116209784
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Malathion, an extensively used organophosphate pesticide, is a vital tool for agricultural pest management [9]. However, its presence in aquatic ecosystems poses significant threats to non-target species like Nile tilapia [10]. Exposure to malathion leads to physiological disruptions, including altered enzyme activity, oxidative stress, and tissue damage in critical organs such as the liver and gills. These adverse effects diminish fish health, impair growth, and lower survival rates [11].

The infiltration of malathion into aquatic habitats disrupts food webs and reduces species diversity, endangering ecological stability. Understanding these impacts is essential for devising strategies to minimize malathion's ecological footprint and safeguard aquatic biodiversity [12]. From this point, the current study was conducted to evaluate the interaction between both malathion and ZnNPs on Nile Tilapia by investigating hepatic damage markers, antioxidant status and proinflammatory markers.

Material and Methods

Chemicals

Zinc oxide nanoparticles (Product No. 544906-50G, <100 nm particle size, ≥97% purity, surface area ~15–25 m²/g) and malathion (Product No. 1374408-500MG, ≥95% purity) were purchased from Sigma-Aldrich (USA). All commercial kits for biochemical and antioxidant defense system were supplied from Biodiagnostic, Co, Egypt. Gene expression analysis kits were supplied from Intronbio, South Korea.

Experimental Design

The experiment was conducted on sixty adult monosex Nile Tilapia (65±10 g) that were bought from local Farm, Al-Manzala Lake, Dakhlia, Egypt and were kept on 60L aquariums of dechlorinated tap water and water quality parameters were monitored using Senso Direct 150 (Lovibond Germany). Water conditions were maintained at 22±1°c, pH (7.4–7.8), and dissolved oxygen levels (6±0.5 ppm).

Fish were divided into four groups in duplicate, each group was comprised of fifteen fish: Group I (Control), Fish was maintained in dechlorinated tap water and fed a commercial basal diet, Group II, Fish was exposed to malathion (3mg/L) [12]. Group III, Fish was exposed to ZnNPs (2mg/L) [13]. Group IV, fish were exposed to zinc oxide nanoparticles and malathion. After thirty days, blood samples were collected from the caudal vein after 30 days, allowed to coagulate, and centrifuged at 5000 rpm for 10 minutes. Serum samples were stored at −20 °C for biochemical analyses. After the collection of blood samples, fish were euthanized with MS-222 (400 mg/L).

Liver and gills were harvested on ice, washed with cold saline, dehydrated, and processed as follows: The first part of both liver and gills was homogenized in phosphate-buffered saline, centrifuged (3000 rpm, 4 °C, 20 minutes), and supernatant stored at −80 °C for oxidative stress biomarker analysis, the second part of liver and gills was fixed in 10% formalin, dehydrated in alcohol, cleared in xylene, embedded in paraffin, sectioned (5–7 µm), and stained with hematoxylin and eosin for histopathological examination, and the last part of hepatic tissues was stored in liquid nitrogen for gene expression analysis.

Serum Biochemical Analysis

The collected serum samples were used to determine the activities of ALT and AST [14] as well as the levels of serum total protein [15], albumin [16], and glucose levels [17] that were conducted using commercial kits according to the manufacturer's instructions

Oxidative Stress Biomarkers

The homogenized tissues of the liver and gills were used to determine the activities of glutathione - S transferase (GST) [18], catalase (CAT) [19] and the levels of lipid peroxidation, malondialdehyde (MDA) [20] and reduced glutathione (GSH) levels [21] according to manufacturer instructions

Gene Expression analysis of immune genes

The RNA extraction process was performed from liver tissue using Trizol reagent according to the manufacturer's instructions [22]. An equivalent of one microgram of RNA was transferred to cDNA using a commercial kit supplied by Intron-bio, South Korea. Quantitative real-time polymerase chain reaction was conducted using a sybr-green master mix that was provided by Intron-bio, South Korea. Gene expression analysis was performed on transform growth factor-beta (TGF-β), interleukin-8 (IL-8) and Interleukin -1β using [23]. Threshold cycle (ct) and melting curve were collected from real-time PCR machine (Pikoreal; Thermo Scientific) and the ct values of all genes were normalized against control and house-keeping gene (subtraction of ct values of the quantified genes in each group from β-actin, then the values of control group was subtracted from given values of studied genes) to determine the equation of fold expression in quantified genes ($2^{-\Delta\Delta ct}$ method) according to [24].

Statistical Analyses

Statistical analyses were conducted using SPSS software v.23 for analysis of all biochemical parameters, where the values were expressed as mean accompanied with standard error. One way analysis of variance was used for comparison between means employing Duncan as a post-hoc test, where significant level was kept at 0.05 [25]. Data

were plotted as graphs, where each mean containing different letters was showing a significant difference.

Results

Serum hepatic damage markers

A significant decrease in total protein in serum was observed in Malathion (GII), and Malathion and ZnNPs groups (GIV) when compared to the normal control group (GI). On the other hand, a significant increase in serum total protein concentration was observed in ZnNPs group (GIII) in comparison to normal Malathion (GIII) and Malathion and ZnNPs groups (GIV) (Fig.1A). A significant decrease in serum albumin concentration in Malathion (GII) and Malathion and Zinc Oxide (GIV) groups as compared to normal control group (GI). On the other hand, the current result showed a significant increase in serum albumin in ZnNPs (GIII) and Malathion and ZnNPs (GIV) groups as compared to the Malathion group (GII) (Fig. 1B).

The activity of AST was significantly increased in Malathion (GII), ZnNPs (GIII), Malathion and ZnNPs (GIV) groups as compared to the normal control group (GI). Also, groups ZnNPs(GIII) and Malathion ZnNPs (GIV) groups were significantly decreased in AST activity compared to the Malathion group (GII) (Fig.1C). Serum ALT activity was significantly increased in the Malathion (GII) group as compared to other groups. However, ZnNPs (GIII) and Malathion and ZnNPs (GIV) groups were significantly decreased in ALT activity compared to the normal control group (GI) (Fig. 1D). Serum glucose concentration was significantly increase in Malathion group (GII) when compared to other groups. However, glucose concentration was significantly decreased in the ZnNPs group (GIII) when compared to other groups (Fig.1E).

Hepatic and branchial antioxidant and oxidative stress markers

Branchial GSH concentration was significantly decreased in ZnNPs (GIII) as compared to other groups. Also, gills GSH concentration was decreased substantially in Malathion and ZnNPs (GIV) group as compared to Malathion (GII) and normal control (GI) groups (Fig.2A). GST activity was significantly decreased in gills of ZnNPs (GIII) and Malathion and Zinc Oxide (GIV) groups as compared to normal control (GI) and Malathion (GII) groups. On the other hand, GST activity in gills of the Malathion (GII) group was significantly increased when compared to different studied groups (Fig.2B). MDA concentration was significantly increased in gills of Malathion (GII) groups as compared to other groups (Fig. 2C). A significant decrease in branchial CAT activity in Malathion (GII) and Malathion and ZnNPs (GIV) groups as compared to normal control group (GI). On the other hand, branchial CAT activity was significantly increased in ZnNPs (GIII) group as compared to different studied groups (Fig. 2D). Hepatic GSH concentration was significantly increased in Malathion (GII) , ZnNPs(GIII), Malathion and ZnNPs (GIV) groups in comparison with normal control group (GI) (Fig.2E). Hepatic GST activity was significantly increased in Malathion (GII) group when compared with normal control (GI), ZnNPs (GIII), Malathion and ZnNPs (GIV) groups (Fig. 2F). A significant decrease in hepatic MDA concentration in ZnNPs (GIII) and Malathion and ZnNPs (GIV) groups when compared to normal control (GI) and Malathion (GII) groups (Fig. 2G). A significant increase in hepatic CAT activity in Malathion (GII) as compared to all other groups. On the other hand, hepatic CAT activity was significantly decreased in ZnNPs (GIII) group as compared to other groups (Fig.2H).

Hepatic gene expression analysis

A significant increase in TGF-β gene expression in ZnNPs (GIII) and Malathion and ZnNPs (GIV) groups when compared to the normal control group (GI). On the other hand, TGF-β gene expression was significantly decreased in the Malathion group (GII) when compared to different studied groups (Fig. 3A). The obtained results revealed a significant down-expression of IL1-B gene expression level in liver tissue in Malathion and ZnNPs group (GIV) in comparison with the normal control group (GI) (Fig. 2B). The obtained results revealed a significant over-expressed of IL-8 gene expression level in liver tissue in Malathion group (GII), ZnNPs group (GIII) and Malathion and ZnNPs group (GIV) in compared to the normal control group (GI). However, the expression level of IL-8 gene in treated groups ZnNPs group still over than Malathion group (GII) and the Malathion and ZnNPs group (GIV). On the other hand, Malathion and ZnNPs group (GIV) significantly decreased IL-8 gene expression as compared with Malathion group (GII) and ZnNPs group (GIII) (Fig.2 C).

Branchial histopathological examination

Microscopic pictures of H&E stained gill sections showing well organized primary and secondary gill lamellae in control group (Group I) and group received zinc nanoparticles (Group II) Gill sections from malathion intoxicated group (group III) showing severe pathological lesions including: lifting up of epithelial cells lining secondary gill lamellae (headed black arrow), congestion (red arrow) and edema (black arrowhead) in primary gill lamellae, variable degrees of hyperplasia of epithelial cells lining primary gill lamellae (dashed black arrow) leading to fusion of secondary gill lamellae (double headed arrow), deviation and deformity of cartilage (red arrowhead). Gill sections from fish intoxicated with malathion and treated with zinc nanoparticles (Group IV) showing preserved architecture primary

and secondary gill lamellae. Low magnification: X:100 bar 100 and high magnification: X:400 bar 50.

Hepatic histopathological examination

Microscopic pictures of H&E stained liver sections showing well organized architecture, normal vacuolated hepatocytes (H) containing considerable amount of glycogen and normal intrahepatic pancreatic cells (PC) in Group I and III. Liver sections from Group II showing severe pathological lesions including: loss of normal architecture، disappearance of glycogen content from hepatocytes (H) (headed black arrow(in some areas while fat vacuoles appear in hepatocytes in other areas (dashed black arrow), congestion (red arrow), severely vacuolated (red arrowhead) and necrotic (black arrowhead) pancreatic cells having small shrunken and pyknotic nuclei. Liver sections from treated group IV showing preserved architecture with mild decrease of glycogen content of hepatocytes (H) in some area (headed black arrow) and activation of melanomacrophage centers (black arrowheads). Low magnification: X: 100 bar 100 and high magnification: X: 400 bar 50.

Discussion

The TGFβ1 gene encodes the transforming growth factor beta-1 (TGFβ-1) protein, which plays a crucial role in regulating cellular processes such as growth, proliferation, differentiation, motility, and apoptosis [26]. The results demonstrated a significant increase in TGFβ1 gene expression in the GIII (ZnNPs) and GIV (Malathion + ZnNPs) groups compared to the normal control (GI) group. Malathion's capacity to create a pro-inflammatory environment aligns with its established role in leukemogenesis [27]. Moreover, TGFβ-1 acts as both a pro-inflammatory and anti-inflammatory cytokine, consistent with the increased expression observed in the GIII and GIV groups. These findings are supported by Zhu et al., who reported that elevated dietary ZnNPs levels in weaned piglets enhanced anti-inflammatory TGFβ-1 expression, improving intestinal health and growth performance [28].

In contrast, this study revealed a significant downregulation of IL-1β gene expression in liver tissue in the GIV group compared to the GI group. This aligns with prior research showing that organophosphate pesticides, including malathion, alter cytokine and chemokine expression [29]. Although the mechanism behind organophosphate-induced inflammation remains uncertain, it is suggested that these chemicals interact directly with inflammatory cells, triggering the release of mediators. Malathion exposure has been shown to stimulate macrophages, generate reactive oxygen species (ROS), and release histamine from mast cells and basophils. In this study, Nile tilapia exposed to malathion + ZnNPs displayed reduced IL-1β levels, underscoring ZnNPs protective role in mitigating inflammatory responses. This aligns with previous findings that ZnNPs influence inflammatory pathways via NF-kappa signaling [30].

In the current study, ZnNPs succeeded in the elevation of IL-8 expression which returned to the basal level in combination with malathion. ZnNPs incriminated in the over-production of IL-1β and IL-8 and suppressed levels of apoptosis [31]. IL-8 is considered as a proinflammatory cytokine that shares a role in immune response and inflammatory conditions in Nile tilapia [32]. From the current results, it is obvious that the interaction of both ZnNPs and malathion achieved a significant reduction in the expression level of IL-8 when compared to the ZnNPs group.

The results revealed a significant reduction in the levels of serum total protein and serum albumin levels after intoxication with malathion in fish which was found due to cellular destruction in kidneys as well as proteolysis and increased catabolism of proteins [33], that indicated the failure of liver for the biosynthesis of hepatic albumin [34]. It is recorded that supplementation of zinc nanoparticles (10 ppm) was incriminated in improving serum albumin levels and albumin/globulin ratio [35]. The interaction of malathion with ZnNPs was found to significantly improve serum albumin levels and serum total proteins. Surprisingly, higher levels of ZnNPs up to 50 ppm were found to produce a significant production of albumin increasing serum total protein levels [36] in Asian catfish. Glucose is important as a main source of energy in aquatic animals, where a significant increase in the levels of glucose can be initiated as a result of the increase of stressors exposed to Nile tilapia [34] and [37]. Serum aspartate transaminase (AST) and serum alanine transaminase (ALT) activities are reliable indicators of chemical toxicity. This study revealed a significant increase in AST and ALT activities in the GII (Malathion), GIII (ZnNPs), and GIV (Malathion + ZnNPs) groups. The elevated levels of protein metabolic enzymes may be attributed to hepatic damage caused by malathion exposure. Malathion, being lipophilic, accumulates in cellular membranes, generating free radicals that result in hepatic injury, consistent with earlier studies [38]. Increased AST and ALT activities were also noted in ZnNPs-treated groups, suggesting cellular damage and oxidative stress in liver cells due to the nanoparticles' small size and chemical properties [39].

Glutathione S-transferase (GST) activity and reduced glutathione (GSH) levels are vital enzymes that protect tissues against oxidative stress. They function as reducing agents during oxidative reactions and are crucial for maintaining cellular redox balance [40]. In this study, exposure to malathion or ZnNPs elevated GSH levels and GST activity in the liver cells of Nile tilapia, while contrasting effects were noted in the gills. GSH serves as a secondary defense mechanism by preserving cells in

their reduced state, offering protection against oxidative damage [41].

Catalase (CAT), an enzyme that scavenges hydrogen peroxide (H_2O_2), plays a critical role in the first line of antioxidant defense [42]. While CAT activity significantly increased in the liver, the accumulation of H_2O_2 indicated oxidative damage in liver tissue. Conversely, in the gills, CAT activity was enough to neutralize excess H_2O_2. The observed increase in CAT activity underscores its role in mitigating ROS damage by converting H_2O_2 into water and molecular oxygen [43].

Additionally, elevated malondialdehyde (MDA) levels in the gills indicated lipid peroxidation, reflecting oxidative damage. The accumulation of H_2O_2 in the gills, but not in the liver, suggests that the gills were more susceptible to oxidative stress caused by malathion and ZnO nanoparticles.

Histopathological analysis revealed extensive damage in the gills and hepatic tissues, including severe pathological lesions in group II which was alleviated by supplementation of zinc nanoparticles. Higher doses of zinc up to 70 ppm may cause extensive damage of tissues [44]. In the current study, the administration of low concentration of zinc nanoparticles achieved alleviation of toxic effect of malathion, however, one of the limitation of the current study was further extension of the experiment period of low zinc nanoparticles can provide more protection and improvement in antioxidant status

Conclusion

The interaction between malathion and ZnNPs in Nile tilapia would significantly improve hepatic damage markers, decrease the levels of lipid peroxidation and modulate the expression profile of immune genes that could be applied efficiently in aquaculture to alleviate pesticide pollution, but require supplementation for long period.

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Not applicable.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All experimental procedures were approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Mansoura University, and conducted in accordance with the institutional guidelines for the care and use of laboratory animals (Approval No. MU-ACUC-2025-014)..

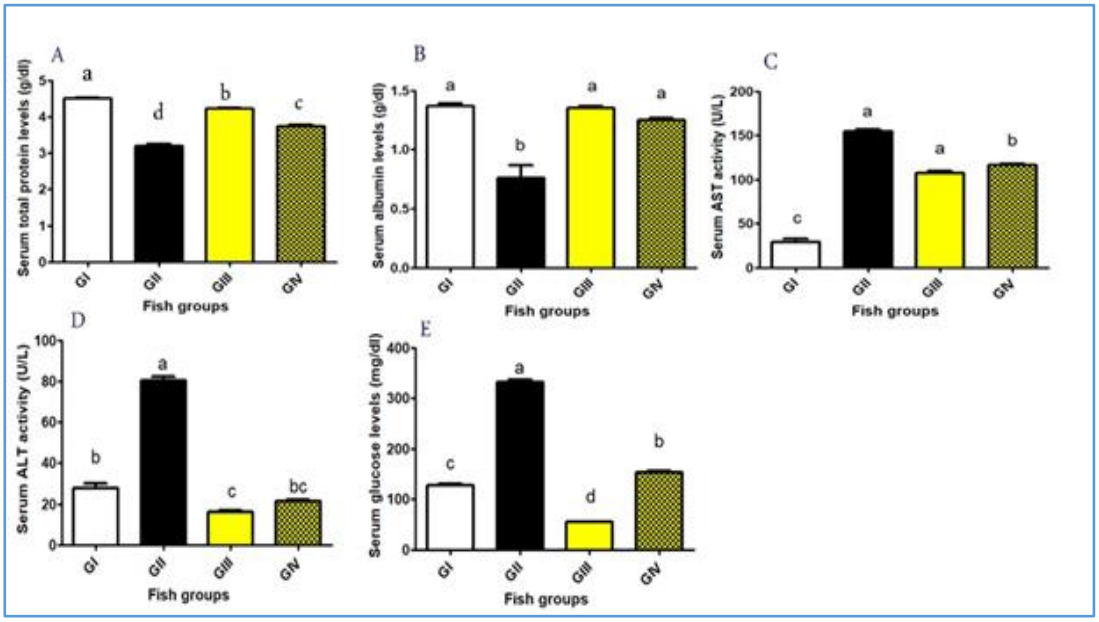

Fig. 1 Serum biochemical parameters of Nile Tilapia exposed to Malathion and treated with zinc nanoparticles; (A) serum total protein; (B) serum albumin level; (C) Serum AST activity; (D) Seum ALT activity; (E) serum glucose level

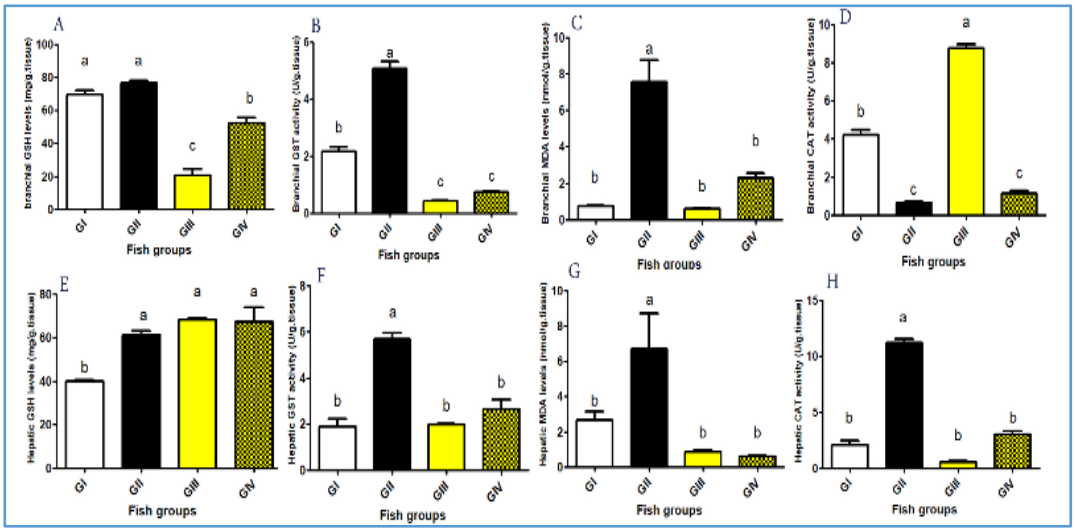

Fig. 2. Branchial and hepatic antioxidant stress markers in fish intoxicated with Malathion and treated with zinc nanoparticles; (A and E) GSH levels; (Band F) GST activity; (C and G) MDA levels and (D and H) CAT activity

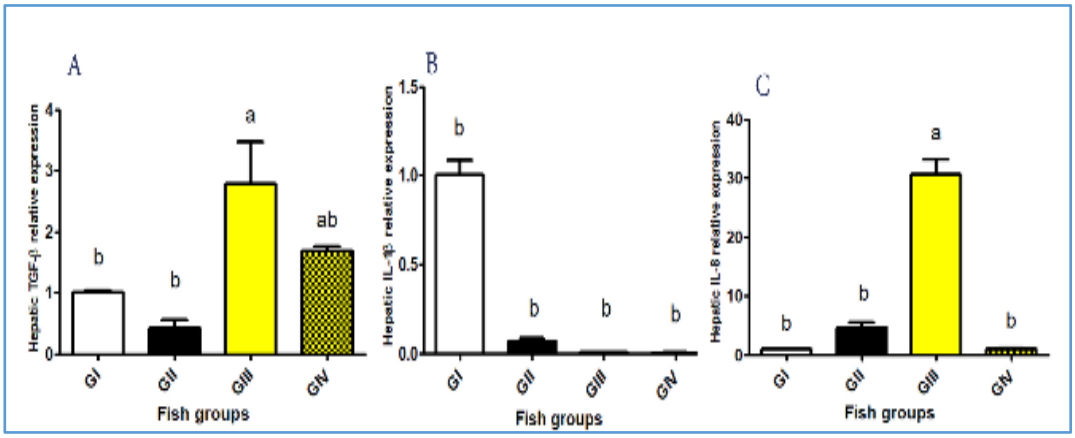

Fig. 3. Hepatic immune genes expression in rats exposed to Malathion and treated with zinc nanoparticles; (A) hepatic TGF Beta relative expression; (B) Hepatic IL1-Beta relative expression; (C) Hepatic IL8- expression

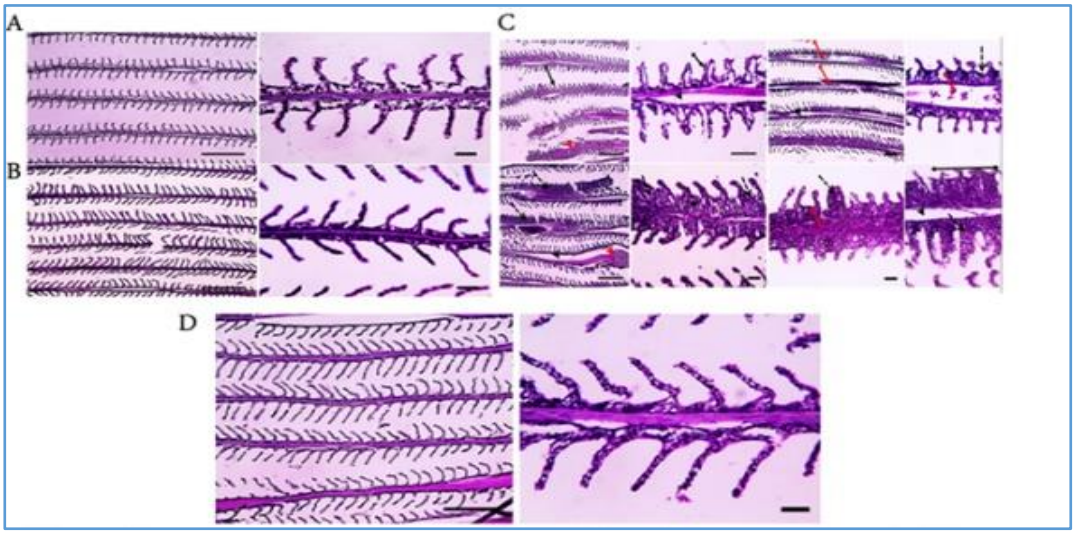

Fig. 4. Microscopic examination of gills in Nile Tilapia exposed to Malathion and treated with Zinc nanoparticles; (A) group I; (B) group II; (C) group III; (D) group IV; . H & E Stained, 400x, bar 50.

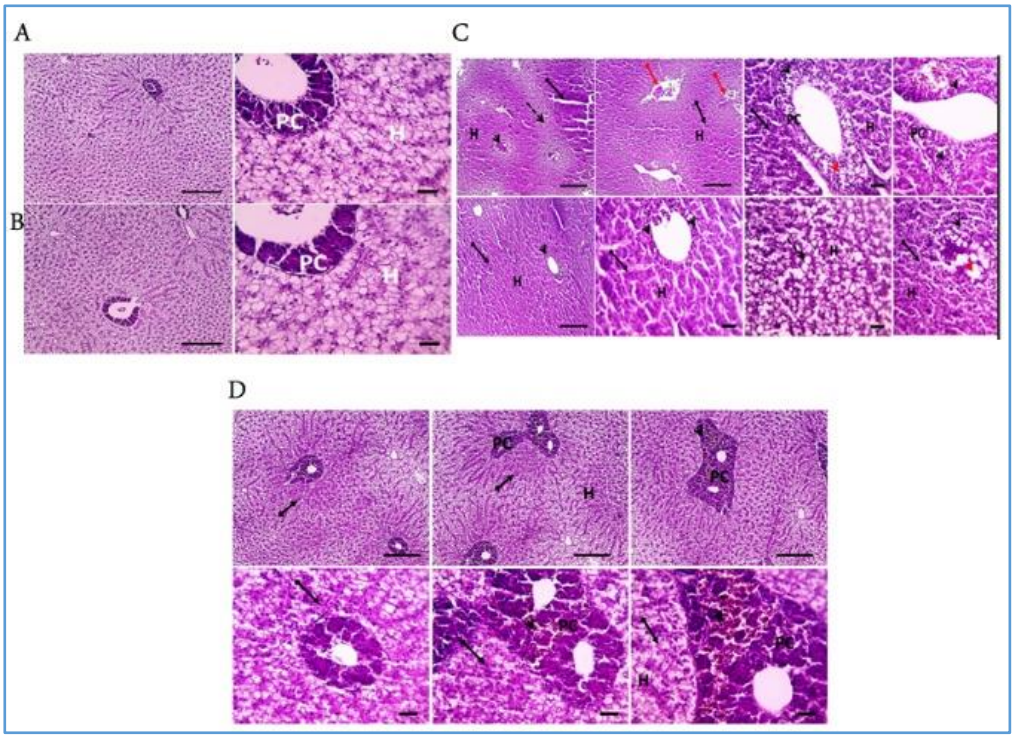

Fig. 5. Histopathological examination of liver in Nile Tilapia exposed to Malathion and treated with Zinc nanoparticles; (A) group I; (B) group II; (C) group III; (D) group IV; . H & E Stained, low magnification 100x, high magnification400x and bar 50.

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تفاعل جزيئات الزنك النانوية مع الملاثيون فى البلطي النيلي

نهال القهوجى[١] و محمد العدل[١] و جهاد السيد [١] و نفين عبد الخالق[٢]

[١] قسم الكيمياء الحيوية و البيولوجيا الجزيئية، كلية الطب البيطري، جامعة المنصورة، مصر.

[٢] قسم طب الاحياء المائية ، كلية الطب البيطرى ، جامعة المنصورة ، مصر

الملخص

البلطي النيلي (*Oreochromis niloticus*)، وهو أحد الأنواع البارزة في المياه العذبة، تناول هذا البحث تفاعل جزيئات أكسيد الزنك النانوية مع مبيدات الملاثيون على أسماك البلطي النيلي. تلقى البلطي النيلي ٢ ملجم / لتر من جزيئات أكسيد الزنك النانوية و / أو ٣ ملجم / لتر ملاثيون. بعد ثلاثين يومًا، تم جمع عينات المصل لتحديد علامات التلف الكبدي، وحالة مضادات الأكسدة الكبدية والخيشومية، وتحليل التعبير الجيني للجينات المناعية. تم اكتشاف انخفاض كبير في نسبة ALT/AST في جميع المجموعات المعالجة. حسنت الجسيمات النانوية لأكسيد الزنك حالة مضادات الأكسدة وخفضت مستويات بيروكسيد الدهون في المجموعة المسكرة بالملاثيون، مما قلل بشكل كبير من السيتوكينات المسببة للالتهابات. سلطت هذه الدراسة الضوء على إمكانات وخصوصية جسيمات أكسيد الزنك النانوية في التخفيف من سمية الملاثيون في البلطي النيلي مما أعطى رؤى لتحسين سمية المبيدات الحشرية بالجسيمات المعدنية النانوية الفسيولوجية في تربية الأحياء المائية.

الكلمات الدالة: الجسيمات النانوية، البلطي، أكسيد الزنك، الملاثيون، التفاعل.