Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Mitigating Adverse Effects of Lead Acetate on Nile Tilapia Health through Pomegranate Pulp Inclusion: Growth, Morphology, Flesh Quality, Antioxidant Response, Gene Expression, and Apoptosis

Sahar H. El-Nagar [1], Reda Abdallah [2], Abdallah S. Salah [3,4], Amira A. Omar [5], Mustafa Shukry [6], Eldsokey Nassef [7], Doaa A. Dorghamm [8], Mustafa El-Maddaway [9] and Elblehi S. Samar [10].

[1] *Department of Animal Wealth Development, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt; sahr_hamdy@vet.kfs.edu.eg*
[2] *Food Control Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt; ; reda_abdallah@vet.kfs.edu.eg*
[3] *Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh, Egypt.*
[4] *Institute of Aquaculture, University of Stirling, Stirling, United Kingdom*
[5] *Department of Fish Diseases and Management, Faculty of Veterinary Medicine Kafrelsheikh University, Kafrelsheikh 33516, Egypt*
[6] *Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.*
[7] *Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Egypt*
[8] *Department of Biochemistry, Faculty of Veterinary Medicine,* Kafrelsheikh *University, Egypt.*
[9] *Department of Toxicology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.*
[10] *Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Edfina 22758, Egypt.*

Abstract

LEAD (Pb) contamination, caused by human activities, is a prevalent issue in aquatic environment and poses significant risks to fish and human health. Fish, in particular, are highly susceptible to lead accumulation, impeding their growth and overall well-being. This experiment inspected the adverse impacts of lead acetate toxicity on various aspects of Nile tilapia's health, water quality, fish muscle quality, and gene expression. The study also investigated the potential mitigating effects of dietary inclusion with pomegranate pulp. One hundred twenty healthy Nile tilapia of similar sizes were randomly split into four experimental groups: a control group, a group supplemented with pomegranate pulp at 15%, a group exposed to lead acetate 24.4 mg/L (20% of the LC50), and a group exposed to both pomegranate pulp and lead acetate. The fish were fed their respective diets for six weeks, and various parameters were assessed. The findings revealed that lead acetate exposure significantly hindered fish growth and resulted in increased stress biomarkers, leading to liver damage and renal failure. Furthermore, lead acetate negatively affected immune parameters and caused elevated oxidative stress levels in the liver and kidneys. However, inclusion pomegranate pulp effectively alleviated these adverse effects by enhancing antioxidant and immune activities. The study also demonstrated that pomegranate inclusion reduced muscle damage and improved the quality of fish meat. Additionally, it influenced the genes expression associated with antioxidant activity, apoptosis, and detoxification in various organs of Nile tilapia. In conclusion, lead acetate had detrimental consequences on the performance of Nile tilapia, but dietary inclusion with pomegranate pulp proved to be an effective strategy for mitigating these effects. Pomegranate pulp enhances antioxidant defenses, boosts immune response, and improves overall health, thereby reducing the adverse impacts of lead exposure.

Keywords: Pomegranate pulp; lead Toxicity; flesh quality; antioxidant markers; *Oreochromis iloticus*.

Introduction

Tilapia farming, as a form of aquaculture, can significantly contribute to the fight against food insecurity, malnutrition, and poverty in Africa [1]. In the aquaculture sector, Nile tilapia (Oreochromis niloticus) are considered the most significant freshwater fish for commercial production due to its high nutritional value, rapid growth rate, and resilience to infections

*Corresponding author: Sahar Elnaggar, E-mail: sahr_hamdy@vet.kfs.edu.eg.; Tel.: +201099868608
(Received 14/03/2024, accepted 14/05/2024)
DOI: 10.21608/EJVS.2024.276858.1915

[2]. Heavy metal ions, released by industrial and domestic waste, seriously pollute the environment and impact all living organisms, leading to immediate and long-term ecological, biological, physiological, and economic issues [3, 4]. They are persistent contaminants that accumulate in highly structured organisms along the food chain [5]. Cadmium, mercury, lead, and chromium cannot be removed from the body by normal physiological processes, accumulate in live bodies, and produce harmful effects in direct correlation with the amounts when certain levels are exceeded [6]. Heavy metals are the most harmful contaminants found in water, which occur naturally as trace components. They alter the physicochemical properties of water. Fish poisons, illnesses, and death can cause a drop in the quantity and quality of fish stocks [7]. The greatest threat posed by heavy metals is their ability to remain in the food chain [8]. Lead is considered one of the most dangerous heavy metal pollutants in an aquatic habitat, which can build up in fish tissue and ultimately kill the fish [9]. One of its components, lead acetate (LA), is the most hazardous of the many naturally occurring forms of lead [10]. Exposure to multiple lead concentrations slowed fish growth [11]. Moreover, LA causes a significant decrease in total protein, calcium, phosphorus, and magnesium levels, along with reductions in superoxide dismutase (SOD) and glutathione. Additionally, there has been a substantial increase in lipid peroxidation (LPO) levels.[12]. The selection of specific genes such as ALAD, SOD, Caspase-3, GSH, Cytochrome P450, and Metallothionein is vital for examining the protective effects of pomegranate pulp against lead acetate toxicity in Nile tilapia. ALAD is essential for studying lead's impact on heme synthesis, as it is notably inhibited by lead, resulting in toxic build-up and associated effects [13].

Pomegranate peel (PP), a waste product, has more antioxidants than pomegranate juice [14]. According to Orzuaa et al. [15], the PP makes up between 5% and 15% of the overall weight of a pomegranate. It is an appealing prospective dietary supplement for livestock [16]. Because it includes bioactive substances, particularly flavonoids, phenolic acids, and antioxidant polyphenols, mostly of hydrolysable tannins and anthocyanins [17], due to its valuable components, it could be a candidate of choice for potential health advantages [18]. It has been extensively considered for its potent antibacterial and anti-inflammatory activities [19]. Examining this plant product's impacts on fish performance is essential to understanding how it affects fish. Thus, the goal of this study was to investigate the protective effects of adding natural pomegranate peels to the diet on the following parameters: growth performance, muscle quality, water quality, gene expression, and histopathological changes in Nile tilapia (Oreochromis niloticus) exposed to water contaminated with lead acetate.

Material and Methods

Declaration of ethics

The methodologies, animal care, and experimental protocols used in this work were compliant with Kafrelsheikh University's rules and regulations. The Institutional Animal Care and Use Committee of Kafrelsheikh University approved the experimental protocol (KFS-IACUC/133/2021).

Experimental design

A total of 120 healthy Nile tilapia (*Oreochromis niloticus*) were sourced from a fish hatchery in Kafrelsheikh, Egypt. They were transported to the Department of Animal Wealth Development, Faculty of Veterinary Medicine, Kafrelsheikh University, where they were acclimated to laboratory conditions and provided ad libitum feeding for 14 days before the start of the study. Fish of similar sizes (12.15 ± 0.02 g) were then randomly placed into 12 glass aquariums (30 × 40 × 60 cm) with a density of ten fish per aquarium. Peel from pomegranates, purchased from a Kafrelsheikh local market, Egypt, was crushed and added to the basal diet at a concentration of 15%[20] The basal diet formulations were prepared according to the established protocols [21] to meet the *O. niloticus* requirements. Lead acetate [Pb (CH3COO)2] was purchased from Sigma-Aldrich and Merck (Germany), and lead acetate was added to the water at a dose of 24.4 mg/L (20% of the LC50), according to [11]

The aquaria used in our study were provided with constant aeration, dissolved oxygen (DO) at 6.5 ± 0.5 mg/l, and a temperature of (22 ± 3°C). Acclimated *Oreochromis niloticus* were allotted into 4 experimental groups, each with 3 replicates (10 fish/replicate), that were treated for six weeks in this way: Group 1 (CTR): control group fed the basal diet without any inclusion; Group 2 (Pom): fed pomegranate pulp included diet at 15% (Table 1); Group 3 (Pb): treated with lead acetate at a concentration of 24.4 mg/L water; Group 4 (Pom+Pb): treated with a mixture of pomegranate pulp included diet at 15% and Lead acetate at a concentration of 24.4 mg/L water. Fish fed the experimental diets twice daily (8:00 a.m. and 4:00 p.m.) up to the satiation level for six weeks. Fish were fed experimental diets at a daily rate of 3% of total biomass till the end of experimental period

Measures of growth performance

Growth performance was evaluated after six weeks. All fish were weighed using a digital balance (DTF, USA; HZ & HUAZHI), and calculations were made using the following equations: Body weight gain (BWG; %) = (FBW-IBW) ×100/IBW. Feed conversion ratio (FCR) = dry feed intake (FI; g) / live weight gain (g). Where FBW is the final body weight (g), and IBW is the initial body weight (g)[22, 23].

Water quality analysis

Throughout the experimental period, regular monitoring of water physicochemical parameters was conducted. Total ammonia-nitrogen levels were measured calorimetrically, and the unionized ammonia (UIA) was derived from the pre-estimated total ammonia-nitrogen using formulas adapted [24]. Additionally, water temperature, pH, and dissolved oxygen (DO) were measured using specific instruments including a thermometer, a portable digital pH meter (Martini Instruments Model 201/digital), and a waterproof portable dissolved oxygen meter (Hanna waterproof Model IP67).

Histopathological analysis and lesions scoring

After administering anesthesia, tissue samples from the gills, intestine, hepatopancreas, kidney, and spleen were obtained. These samples were promptly cut into approximately 0.5 cm^3 pieces and fixed in Bouin's solution for 18–24 hours. Subsequently, after being rinsed in phosphate-buffered saline, the samples were fixed for at least 24 hours in a 10% phosphate-buffered formalin solution (pH 7.4). The standard paraffin embedding technique was then applied to the fixed tissue specimens. Using the protocol described by [28], Paraffin block slices (5 µm thick) were mounted on slides and stained with Mayer's hematoxylin and eosin (H&E). The stained sections were examined using a light microscope (Leica, DM500) and images were captured using a digital camera (EC3, Leica, Germany). Semi-quantitative lesion scoring was employed to evaluate the severity of histopathological alterations found in the treated groups' spleens, kidneys, hepatopancreas, and gills. Histological assessment was performed at a magnification of 100×, with five slides from five different fish per group examined. The extent of histopathological changes was graded as mild (+) when affecting less than 25% of the section's area, moderate (++) when affecting 25–50% of the section's area, and severe (+++) when affecting more than 50% of the section's area, based on the methodology described by [25].

Muscle quality assessments

The measurements of pH, total volatile base nitrogen (TVB-N), and thiobarbituric acid reactive compounds (TBARS)

The pH measurement involved homogenizing 5 g of fish muscles with 10 mL of distilled water, and then using a pH meter (Jenway 3505 pH Meter, United Kingdom) the pH of the samples was measured. Total volatile base nitrogen (TVB-N) was obtained by precipitating proteins with the addition of trichloroacetic acid (TCA), and then measured using the Micro Kjeldahl method, with values measured in mg N/100 g of fish [26]. Using the procedure outlined by [25], malondialdehyde (MDA) was measured using the thiobarbituric acid reactive substances (TBARS) test in order to evaluate oxidative stability, with TBARS identified by phospholipid and lipid-associated protein precipitation. Using a 7.8 conversion factor, the reading from the spectrophotometer (UNICAM969AA Spectronic, USA) was obtained at 532 nm, converting mg of malondialdehyde to kg of food. All measurements were carried out in triplicate.

Lead residue measurement

The lead concentration in the fish muscle tissue was analyzed using a method outlined by [27]. This involved weighing 10 grams of wet muscle tissue for each fish sample 7 fishes per replicate for each group, placing it into a crucible, and subjecting it to overnight heating at 500°C in a furnace. After cooling to room temperature, the tissue was broken down by heating it on a hot plate at 80°C after adding 2 mL of strong nitric acid (65%) and 20 mL of diluted hydrochloric acid (10%) to a polytetrafluoroethylene (PTFE) beaker. A 50 mL sample solution was then made by filtering the resultant mixture through 0.45 µm pore-size filter paper. The lead (Pb) content was then determined using an atomic absorption spectrophotometer (UNICAM969AA Spectronic, USA), with measurements taken at a wavelength of 217 nm.

Gene expression

The gene expression analysis was conducted using real-time quantitative polymerase chain reaction (RT-qPCR) with gene-specific primers for β-actin and GAPDH as internal controls for normalizing gene expression data. Using Trizol (iNtRON Biotechnology) reagents, total RNA was extracted from liver, muscle, kidney, and gill samples from 7 fishes per replicate for each group following the manufacturer's instructions. By using Nanodrop (Quawell, USA), the concentration and purity of the extracted RNA were assessed, and 2% agarose electrophoresis was used to verify the results. Subsequently, 2 µg of total RNA was transcribed using a cDNA synthesis kit (Bioline, UK) according to the manufacturer's instructions. The real-time PCR reactions were carried out in 20 µL reaction mixtures, including 2 µL cDNA, gene-specific primers (0.5 µM each), and 10 µL of SYBR real-time PCR amplification using the SensiFast SYBR Lo-Rox kit (Bioline). The thermal cycling conditions involved an initial denaturation at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for one minute. The gene expression analysis was performed in triplicate to ensure accuracy. Use data management ($2^{-\ \Delta\Delta CT}$) [28].

Histomorphometry investigation

Histological analysis of the hepatopancreases, kidneys, and spleens was conducted using H&E-stained sections from a single organ/fish, with a total of 5 sections per group. After that, digital images were assessed using image analysis software (National Institute of Health, Bethesda, MD, USA; Im-

age J 1.47v). To quantify the frequency (number) and area percentage (%) of MMCs, ten images from each section at x 100 magnification were analyzed for each experimental group [29].

Data analysis

The data were ananlyzed using GraphPad Prism 8.0. The parametric data was compared using a one-way analysis of variance (one-way ANOVA), and the multiple means between groups were compared using a Tukey post-test. The threshold for statistical significance was set at $p < 0.05$.

Results

Growth performance parameters

After six weeks of treatment of tilapia with a pomegranate pulp-included diet and/or lead acetate, the final body weight (FBW) was drastically raised ($P < 0.05$) in group 2 (Pom) linked to group 3 (Pb) and group 4 (Pom+Pb), while no considerable differences between the group 2 (Pom) and the group 1 (CTR). There were no substantial changes in the body weight gain (BWG) between the (pom) and (CTR) groups; however, there was a considerable rise ($P < 0.05$) in the (Pom) one when linked to the (Pb) and (Pom+Pb) (Table 3). There were no notable differences in the feed conversion ratio (FCR) between (CTR, Pom, and Pom +Pb) groups with lower values than the (Pb) group, where the FCR significantly increased ($P < 0.05$) when contrasted with the other categories, as displayed in Table 3.

Water quality parameters

In terms of water physicochemical characteristics, a noteworthy decrease in ammonia levels was observed in group 2 (Pom) and group 4 (Pom+Pb) compared to group 3 (Pb) ($P < 0.05$), as depicted in Figure 1A. Conversely, no significant differences were noted between the (Pom) and (Pom+Pb) groups. Regarding water pH, there were no significant disparities between the (Pom) and the (CTR) groups, while a substantial decrease ($P < 0.05$) was observed in the (Pom+Pb) group compared with the (Pb) group, as illustrated in Figure 1B.

Muscle quality parameters

pH, TVB-N, and TABRS in muscle

Physicochemical (pH values) and chemical (TVB-N as mg nitrogen/100 g sample and TBARS as mg malonaldehyde/kg sample) muscle quality parameters are presented in Table 4. The pH value is one of the essential physicochemical characteristics used to assess sea foods' quality and shelf life. The fish group fed on a diet supplemented with pomegranate Pulp (15% in the (Pom) group which had the highest value (6.26 ± 0.07), with a meaningful variance ($p < 0.05$) to the (Pb) group reared in water polluted with lead acetate, which had the lowest pH value (5.96 ± 0.04), followed by the (Pom+Pb) group (6.07 ± 0.02) (Table 4). Fish samples of the (Pom) group supplemented with pomegranate peel showed significantly lower ($p < 0.05$) TVB-N content (7.88 ± 0.47) compared to the (CTR) group (11.98 ± 0.15) and fish samples of the (pb) group (21.14 ± 0.82), which showed the highest value, followed by fish samples of the (Pom+pb) group (14.46 ± 0.52) (Table 4). Results obtained for TBARS levels (mg of malondialdehyde/kg sample) as a quality parameter detecting lipid oxidation in fish muscle concerning TVB-N values displayed substantial alterations ($p < 0.05$) in the fish samples of the (Pom), recording a lower level (0.15 ± 0.02 mg MDA/kg) in comparison with fish samples of the (pb) group, which recorded the highest value (0.42 ± 0.02 mg MDA/kg), tracked by fish samples of the (Pom+pb) group (0.30 ± 0.01 mg MDA/kg) (Table 4).

Lead muscle residue

As displayed in Table 4. Lead residue in muscle showed that the (Pom) group had the lowest level of Pb residue (0.18 ± 0.02) as associated to the (Pb) group, which showed the highest quantities of Pb residue (0.55 ± 0.03). The (Pom+Pb) group showed a significant decrease (0.40 ± 0.01) in Pb residue as compared to the (Pb) group.

Gene expression

The mRNA gene expression profiles of ALAD, SOD, GSH, Caspase-3, Cytochrome P450, and Metallothionein in the liver, muscle, kidney, and gills of Nile tilapia fish across different experimental groups were visually represented in Figure 2. Notably, the liver, muscle, kidney, and gills of the Pom+Pb group exhibited a significant ($p < 0.05$) increase in ALAD RNA gene expression compared to the CTR group and the Pb group, which showed a significant ($p < 0.05$) decrease in gene expression. In terms of SOD and GSH mRNA gene expression, a significant ($p < 0.05$) increase was observed in the liver, muscle, kidney, and gills of the Pom group, followed by the Pom+Pb group compared to the Pb group and the CTR group. Furthermore, the mRNA gene expression of Caspase-3, Cytochrome P450, and Metallothionein displayed a significant ($p < 0.05$) increase in the liver, muscle, kidney, and gills of the Pb group compared to the Pom+Pb group and a significant ($p < 0.05$) increase compared to the CTR and Pom groups.

Histopathological examination and semi-quantitative lesions scoring

Figures 3-6 describe in detail the histomorphological observations of H&E-stained sections from the kidneys, spleens, gills, and hepatopancreases of the CTR, Pom, Pb, and Pom+Pb experimental groups. An overview of the severity of histopathological changes seen in the tissues under examination is shown in Table 5.

Normal histological features in gills, including a normal gill arch and secondary lamellae perpendicu-

lar to the filament, were seen In the CTR and Pom dietary-supplemented fish (Figures 3A, 3B). Conversely, Pb-treated fish exhibited significant epithelial lifting, curling, hyperplasia, and fusion of secondary lamellae, as well as filamentous clubbing, goblet cell hyperplasia, and telangiectasis, along with lamellar congestion and interstitial edema (Figures 3C-3H). In the Pom+Pb-treated group, a noticeable decrease in the magnitude and distribution of the observed lesions in the Pb-treated group was evident, though some changes persisted (Figure 3I, Table 5).

Hepatopancreases The livers of CTR and Pom dietary-supplemented fish exhibited a typical parenchymal arrangement and normal pancreatic structure, while Pb-treated fish displayed disrupted tissue organization, hepatocellular degenerative changes, vacuolation, necrosis, and inflammatory cell infiltration (Figures 4A-4H). In the Pom+Pb-treated group, a marked improvement in hepatic histology was noted, with a reduction in the severity and distribution of previously observed lesions (Figure 4I, Table 5).

Kidneys The kidneys of CTR and Pom dietary-supplemented fish displayed normal histological structure, but in Pb-treated fish, tubular epithelial attenuation and necrosis, as well as glomerular necrosis, vascular congestion, and increased Melano macrophage centers (MMCs) were observed (Figures 5A-7E). Conversely, the Pom+Pb-treated group exhibited significant improvement in renal tissue structure, with a decline in the degree of severity and pattern of previously detected lesions in the Pb-intoxicated group (Fig. 3F, Table 5).

Spleens The spleens of CTR and Pom dietary-supplemented fish showed normal histological structures, while Pb-treated fish displayed lymphocytic necrosis, fibroblastic proliferation, and disorganized Melano macrophages in the splenic parenchyma (Figures 6A-8E). The spleen of the Pom+Pb-treated group exhibited a decrease in the seriousness and distribution of previously observed lesions in Pb-treated fish, although some changes persisted within control limits (Figure 6F, Table 5).

MMCs Frequency and area %

Hepatopancreas, kidney, and spleen tissue sections stained with Hematoxylin and Eosin (H&E) are quantitatively analyzed in Table 6, with a focus on the frequency and area percentage of Melano macrophage centers (MMCs). The average number and area % of MMCs did not significantly change in the dietary-supplemented CTR or Pom fish. However, the Pb-intoxicated fish exhibited a notable increase in these parameters compared to the CTR group values. Conversely, the Pom+Pb-treated group showed a slight rise in the average number and area percentage of MMCs compared to the CTR group, but these values decreased substantially compared to the Pb-treated group.

Discussion

As a member of the Punicaceae family, the Long-Pomegranate (Punica granatum) gets its name from the Latin word for "granular apple" (Malum granatum) [30]. Although the pomegranate tree is indigenous to Iran and India, its cultural influence has spread to the Mediterranean and the southwestern United States [31]. Pomegranate greatly ($P<0.05$) enhanced Nile tilapia growth performance in terms of final weight, weight gain, and feed conversion ratio [32] which was in agreement with our results.

Damage to aquatic organisms' DNA, physiology, and biochemistry, as well as their behaviour, can result from heavy metal contamination [33]. Heavy metal contamination primarily affects fish [34] in order to assess the potential genotoxic impacts of contaminants in water [35]. During the investigation, notable elevations in both ammonia and pH levels were observed in the (Pb) group throughout the study. This occurrence is likely attributed to the introduction of lead acetate, which has the effect of raising the alkalinity. According to [36], The water's physiochemical composition was altered as a result of the introduction of lead nitrate and the release of metabolic wastes, fish fecal matter, and unconsumed food materials into the surrounding water. Pomegranate peel can lower ammonia and pH, according to the current study. With its high affinity to adsorb ammonium ions and maximum ammonium removal capability at low pH, pomegranate peel powder offers a cost-effective solution for ammonium removal [37]. In polluted areas, pomegranates may act as a bio-adsorbent of high quality, removing heavy metal ions [38]. Evidence suggests that the peel of pomegranate can be used as an adsorbent to draw copper and lead ions out of water [39].

The pH value of muscle is one of the essential physicochemical characteristics used to assess sea foods' quality and shelf life. The Pom group fed on a diet supplemented with pomegranate peel had the lowest Ph value, with a significant difference to the group reared in water polluted with Pb, which had the highest pH value, followed by the Pom+Pb group, which fed on pomegranate peel and was raised in water contaminated with lead. The lowest values in groups that feed on pomegranate may be accredited to that pomegranate peel extracts contain some acids, as Ellagic acid (Ellagitannin, Punicalagin, and Punicalin), which decrease the pH value of muscle [40].

Volatile compounds like trimethylamine, ammonia, and dimethylamine are categorized as bbbb b55xz6c of phenolic compounds in pomegranate peels, which have the ability to interrupt lipid oxidation by donating a hydrogen atom to free radicals [41].

The Bioremediation effect of pomegranate peel on lead muscle residue in the of Nile tilapia was in-

tense; it was found that pomegranate peel lowered the lead residue in the Pom+Pb group in comparison with the Pb group that reared in polluted water with lead. The strong adsorptive capacity of pomegranate for heavy metals such as mercury and lead could be attributed to its essential components, including phenolic compounds such as gallic, ellagic, and caffeic acids, as well as minerals, complex polysaccharides, flavonoids like catechin, epicatechin, and gallocatechin, anthocyanins, condensed tannins, hydrolyzable tannins like ellagitannins and gallotannins, alkaloids such as punigratane, and lignans like isolariciesinol. [42]. Also, [43] revealed that pomegranate is rich in oxygen (48.35%). The abundance of oxygenated surface groups in pomegranate enhances its ability to chelate heavy metals like lead (Pb) [44]. Additionally, [30] observed a significant reduction in the level of Pb in Mugil cephalus flesh samples after treating them with an aqueous pomegranate solution (1 g pomegranate powder per 100 g fish) for 30 minutes. They attributed these findings to the presence of polyphenols, tannins, and flavonoids in pomegranates.

The hepatocytes revealed degeneration and central vein congestion. These findings point to liver injury in the organ that is responsible for excretion, detoxification, and the production of binding proteins like metallothionein. Further evidence of an increase in cellular damage is provided by the metal-binding proteins found within the nuclei of hepatocytes [45]. Comparable data were reported by [46] and [47]. The fish's liver is particularly vulnerable to environmental contaminants because it accumulates these substances at higher levels than other organs [48]. Fish possess a sequestering agent known as metallothionein, which helps bind and store trace elements. However, the process by which these trace elements build up in the liver can reach critical levels, impairing liver function. This can result in the progressive degeneration of liver cells and disruption of their syncytial arrangement [49]. The degenerative process mentioned is accompanying by a reduction in the surface area of liver cells, possibly owing to a surge in intrabiliary fiber-connective tissue. The presence of intercellular spaces indicates significant cell degeneration. Prolonged hepato-cellular injury can ultimately lead to cirrhosis, characterized by the formation of hepatic cords and fibrosis in the peribiliary connective tissue. Similar variations have been observed in the kidneys of Tilapia and other fish species exposed to heavy metals, as documented by [50], and [51], The outcomes of the present investigation are in agreement with these conclusions.

Toxic compounds found in environmental contaminants can easily penetrate the gills of aquatic creatures. The gills are particularly susceptible since they are in direct contact with these toxins. The contact between the gills and environmental pollutants enables the absorption of harmful compounds into the organism's system. However, the absorption process is mitigated by certain factors. Firstly, the improved permeability of the gill epithelium to water and ions reduces the absorption of toxic chemicals. This increased permeability allows for the efficient exchange of water and ions, which can help flush out or dilute pollutants. Secondly, the ion exchange activity in the chloride cells, a crucial component of gill function, is inhibited by environmental pollutants. This inhibition further limits the uptake of harmful compounds by the gills. These defense mechanisms aim to protect aquatic organisms' gills and overall health from the detrimental effects of toxic chemicals [52]. Exposure of fish to lead acetate has been found to cause several histological changes in their tissues. These changes include hypertrophy, which refers to an increase in cell size.

Additionally, the epithelial linings detach from the surfaces of secondary lamellae, and there is degeneration of the lamellar epithelium in certain areas. It is worth noting that related scrutiny have been reported in other freshwater fish species when exposed to lead. These findings suggest that lead exposure can consistently affect fish histology across different species[53]. The primary cause of cellular degeneration in gill filaments is often attributed to O_2 deficiency from gill toxicity. When the gills are exposed to toxic substances, the oxygen exchange process is disrupted, leading to insufficient oxygen supply to the gill tissues. This oxygen deficiency can lead to cellular degeneration and damage in the gill filaments [54]. The current research study provides evidence of pathological alterations in fish subjected to lead acetate treatment, specifically observing degeneration and vacuolation of hepatocytes. This hepatocyte vacuolation can lead to inhibition of protein synthesis, depletion of energy, and aggregation of microtubules [55]. These findings suggest a potential imbalance between synthesizing substances within parenchymal cells and their release into the circulatory system [56]. The damage to the hepatopancreas is characterized by the loss of contact between hepatocytes and pancreocytes, leading to atrophy.

The findings have been replicated by [57]. In the present study, the widespread occurrence of this pathological condition may be attributed to the loss of energy in the detoxification process, resulting in an increase in metabolic byproducts and the aggregation of melanoma macrophages [58]., Similar effects in medaka (Oryzias latipes) exposed to sublethal levels of methyl mercury chloride, in Channa punctatus exposed to arsenic and in Oreochromis mossambicus exposed to cadmium and zinc[59]. Furthermore, the severity of lesions caused by lead acetate showed a positive correlation with its concentration. These results provide more evidence that histological changes in the gills, liver, and hepatopancreas may have contributed to fish mortality during the exposure period.

To rule out any other possible causes of death, further investigations should concentrate on the brain and other organs [46]. On the contrary, the administration of pomegranate has been found to alleviate the histopathological injuries caused by lead toxicity. Pomegranate possesses antioxidant properties, which help counteract the harmful effects of lead and reduce oxidative stress in tissues. Additionally, pomegranate has been shown to enhance the innate immune system [60, 61], restoring tissue health and reducing the severity of histopathological changes induced by lead toxicity.

Conclusion

The study findings indicate that Nile tilapia fed a diet enriched with pomegranate exhibited improved, water quality, and muscle condition, thereby protecting against lead toxicity. The inclusion of pomegranate in the diet resulted in enhanced immune response and antioxidant capacity, which was achieved by modulating the mRNA gene expression of ALAD, SOD, GSH, Caspase-3, Cytochrome P450, and Metallothionein in the liver, muscle, kidney, and gills of the fish. Consequently, the detrimental effects of lead acetate were mitigated, leading to improvements in tissue pathology caused by lead-induced alterations. However, further research is necessary to explore the immunostimulatory effects of pomegranate on fish immune systems, considering different species and the virulence level of pathogens.

Funding statement: there is no funding.

Informed Consent Statement: Not applicable

Data Availability Statement: Data are available upon request

Conflicts of Interest: The authors declare that they have no competing interests.

TABLE 1. Basal and pomegranate pulp-supplemented diets and proximate chemical composition (on a dry matter basis).

Item	Basal diet	Pomegranate diet (15%)
Fish meal	10	10
Corn gluten meal	6	6
Soybean meal	41.16	41.16
Soybean oil	3	4.8
Corn grains	23.59	21.79
Rice polishing	15	0
Pomegranate pulp	0	15
Dl methionine	0.19	0.19
Dicalcium phosphate	0.51	0.51
Salt	0.25	0.25
Premix	0.3	0.3
Digestible energy	3107	3085
Crude protein	32	32
Crude fat	6.96	6.92
Acid detergent lignin	8.6	10
Lysine	1.83	1.81
Methionine	0.86	0.84
Calcium	0.51	0.58
Available phosphorous	0.45	0.43
Sodium	0.1	0.1

Premix (Egypt pharma): Each 3 kg composed of 5000 mg copper; 5 mg cobalt; 3000 mg iodine; 300 mg selenium; 100,000 mg iron; 30,000 mg manganese; 60,000 mg zinc; 5000,000 IU Vit. A; 500,000 IU Vit. D3; 20,000 mg Vit. E; 4000 mg K3; 8000 mg B1; 8000 mg B2; 8000 mg B6; 8 mg B12; 40,000 mg niacin; 2000 mg folic; 40 mg biotin;20,000 mg pantothenic acid; calcium carbonate ad to 3000 gm.

TABLE 2. Primers are used for RT-qPCR.

Primer Gene	Forward	Reverse	Accession number
GAPDH	GCT GTA CAT GCA CTC CAA GG	ACT CAA ACA CAC TGC TGC TG	NM_001279552.1
ALAD	GTT GTC ACA TCA TCG CTC CC	TCT CTC ACA TCT CGC TCC AC	XM_003440087.4
SOD	GACGTGACAACACAGGTTGC	TACAGCCACCGTAACAGCAG	XM_003449940.5
GSH	CCAAGAGAACTGCAAGAACGA	CCAAGAGAACTGCAAGAACGA	NM_001279711.1
CASP3	CAGGACACGTCATTCCTACAC	GGCTCTTCGTCTGCTTCTGT	XM_005936749.3
Cyto-P450	GCAAATGGCTGCTGCTTGTCA	GTGTATCAAGGGTTCATGCCCT	NM_001279489.1
MT	ACAAACTGCTCCTGCACCTC	CAGCTAGTGTCGCACGTCTT	XM_030425623.1
β-Actin	CAGCAAGCAGGAGTACGATG	TGTGTGGTGTGTGGTTGTTTTG	XM_031749543.1

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ALAD, amino levulinate dehydratase; SOD, superoxide dismutase; GSH, reduced glutathione; CASP3, caspase-3; Cyto-p450, cytochrome P450; MT, metallothionein; β-actin, beta-actin.

TABLE 3. Growth weight and FCR of Nile tilapia fish in the different experimental groups.

Groups	IBW (g)	FBW (g)	BWG (%)	FCR (g)
CTR	12.15 ± 0.024	29.92±0.21^{a}	17.775±0.21^{a}	1.838±0.02^{b}
Pom	12.18 ± 0.003	31.854±0.70^{a}	19.679±0.70^{a}	2.04±0.29^{b}
Pb	12.16 ± 0.03	21.448±1.03^{c}	9.285±1.01^{c}	3.292±0.25^{a}
Pom+Pb	12.12 ± 0.034	26.927±0.83^{b}	14.805±0.84^{b}	1.974±0.09^{b}

CTR = (control group), Pom = (fish group received pomegranate pulp supplemented diet 15%), Pb = (fish group received 24.4 mg lead acetate/L water), and Pom+ Pb = (fish group received pomegranate pulp supplemented diet 15% and 24.4 mg lead acetate/ L water). IBW = Initial body weight (g), FBW = Final body weight (g), BWG= Body weight gain, and FCR= Feed conversion rate. The data represent the mean ± Standard errors (SEM). The data were analyzed using a one-way ANOVA followed by the Tukey posthoc multiple comparisons test. The values with different superscripts (a–c) in the same row indicate significant differences ($p < 0.05$).

TABLE 4. pH, TVB-N, TABRS, and Pb residue in the muscle of Nile tilapia fish in the different experimental groups.

Groups	pH	TVB-N (mg/100 g)	TABRS (mg MDA/kg)	Lead (Pb, ppm)
CTR	6.10 ± 0.02^{a}	11.98 ± 0.15^{c}	0.28 ± 0.02^{c}	0.29 ± 0.01^{c}
Pom	6.26 ± 0.07^{a}	7.88 ± 0.47^{d}	0.15 ± 0.02^{d}	0.18 ± 0.02^{d}
Pb	5.96 ± 0.04^{c}	21.14 ± 0.82^{a}	0.42 ± 0.02^{a}	0.55 ± 0.03^{a}
Pom+Pb	6.07 ± 0.02^{b}	14.46 ± 0.52^{b}	0.30 ± 0.01^{b}	0.40 ± 0.01^{b}

CTR = (control group), Pom = (fish group received pomegranate pulp supplemented diet 15%), Pb = (fish group received 24.4 mg lead acetate/L water), and Pom+ Pb = (fish group received pomegranate pulp supplemented diet 15% and 24.4 mg lead acetate/L water). TVB-N= Total volatile base nitrogen and TABRS= Thiobarbituric acid reactive substances. The data represents the mean ± Standard errors (SEM). The data were analyzed using a one-way ANOVA followed by the Tukey posthoc multiple comparisons test. The values with different superscripts (a–d) in the same row indicate significant differences ($p < 0.05$).

TABLE 5. Lesion scoring in the gills, livers, and spleen tissues of Nile tilapia fish in the different experimental groups.

Type of change	Groups			
	CTR	Pom	Pb	Pom+ Pb
1- Gills				
Lamellae				
- Epithelial				
Hypertrophy	-	-	++	+
Hyperplasia	-	-	+++	++
Lifting	-	-	++	+
- Lamellar fusion				
Focal	-	-	+++	++
Diffuse	-	-	++	+
- Epithelia Narcosis				
primary lamella	-	-	++	+
secondary lamella	-	-	+++	++
- Mucous cells hyperplasia	-	-	++	+
- Rupture of pillar cells	-	-	++	+
- Telangectiasis	-	-	++	+
- Congestion of the branchial blood vessels				
Gill arch				
-Congestion	-	-	+++	++
-Oedema	-	-	++	+
-Infilammatory cells infiltrations	-	-	++	+
2-liver				
Hepatocytes				
-Irregular architecture	-	-	+++	++
-Lipoid vacuoles	-	-	+++	++
-Pyknotic nucleus	-	-	++	+
-Focal area of necrosis	-	-	++	+
-Cellular infiltrates	-	-	++	+
-Intercellular edema	-	-	++	+
Congestion	-	-	+++	++
Activation of MMCs	-	-	+++	+
Portal inflammation	-	-	++	+
Pancreatic cells necrosis	-	-	++	-
Congestion in the pancreatic	-	-	++	+
3. Kidney				
Necrosis of tubular epithelium	-	-	++	+
Tubulointerstitial nephritis	-	-	++	+
Glomerular atrophy	-	-	++	++
Glomerular necrosis	-	-	++	++
Hemorrhage	-	-	+++	++
MMCs activation	-	-	+++	+
4-Spleen				
- Lymphocytic necrosis	-	-	+++	++
-White pulp depletion	-	-	++	+
-Intracellular edema	-	-	++	+
-Vascular congestion	-	-	++	+
-Activation of MMCs	-	-	+++	++

Absent (–) (no lesions); Mild (+) < 25% area of section; Moderate (++) 25–50% area of section; and Severe (+++) > 50% area of section. CTR = (control group), Pom = (fish group received pomegranate pulp supplemented diet 15%), Pb = (fish group received 24.4 mg lead acetate/L water), and Pom+ Pb = (fish group received pomegranate pulp supplemented diet 15% and 24.4 mg lead acetate/L water). The data are shown as the mean ± SEM. The data were analyzed using a one-way ANOVA followed by the Tukey posthoc multiple comparisons test.

TABLE 6. The frequency (number) and the area percentage of MMCs in livers and spleens of Nile tilapia in the different experimental groups.

Groups	Liver		Kidney		Spleen	
	Number	Area-percentage	Number	Area-percentage	Number	Area-percentage
CTR	3.52±.78^{b}	4.6267± 0.71^{c}	4.66±.71^{c}	14.22±0.91^{c}	6.79±1.55^{b}	13.35±0.45^{c}
Pom	3.94±1.0^{b}	5.1467± 1.20^{bc}	5.15± 1.21^{c}	13.84±0.89^{c}	6.53± 0 .84^{b}	15.08±1.36^{c}
Pb	10.35±2.19^{a}	13.14± 2.4^{a}	13.14± 2.44^{a}	26.17±2.04^{a}	16.54± 3.00^{a}	38.13±0.54^{a}
Pom+Pb	7.02±1.26^{ab}	8.48± 1.63^{ab}	8.48± 1.63^{ab}	20.18±4.53 ^{ab}	10.08±0.64 ^{ab}	23.97±3.02^{b}

CTR = (control group), Pom = (fish group received pomegranate pulp supplemented diet 15%), Pb = (fish group received 24.4 mg lead acetate/L water), and Pom+ Pb = (fish group received pomegranate pulp supplemented diet 15% and 24.4 mg lead acetate/L water). The data represent the mean ± Standard errors (SE). The Tukey posthoc multiple comparisons test was used after a one-way ANOVA to assess the results. Values with various superscripts (a-c) in the same row show significant differences ($p < 0.05$).

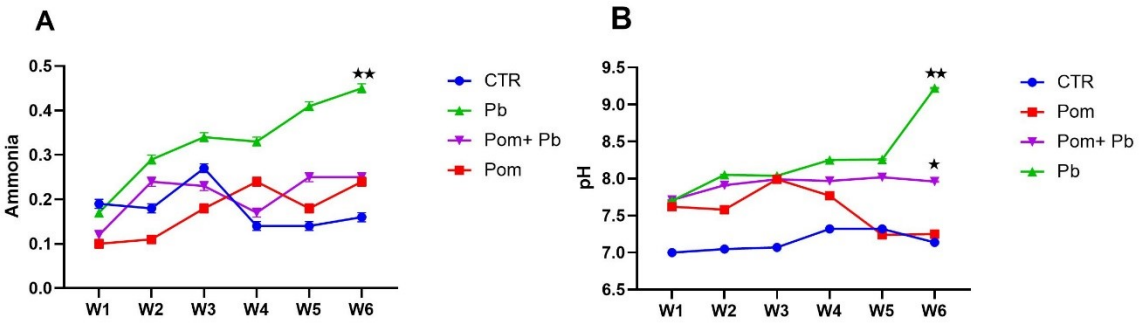

Fig. 1. Ammonia in water and pH of water in the different experimental groups. (A): Ammonia in water. (B): pH of water. CTR = (control group), Pom = (fish group received pomegranate pulp supplemented diet 15%), Pb = (fish group received 24.4 mg lead acetate/L water), and Pom+ Pb = (fish group received pomegranate pulp supplemented diet 15% and 24.4 mg lead acetate/L water). The data are shown as the mean ± SE. The data were analyzed using a one-way ANOVA followed by the Tukey post-hoc multiple comparisons test. The asterisks denote a significant difference (* $p < 0.05$ and ** $p < 0.01$).

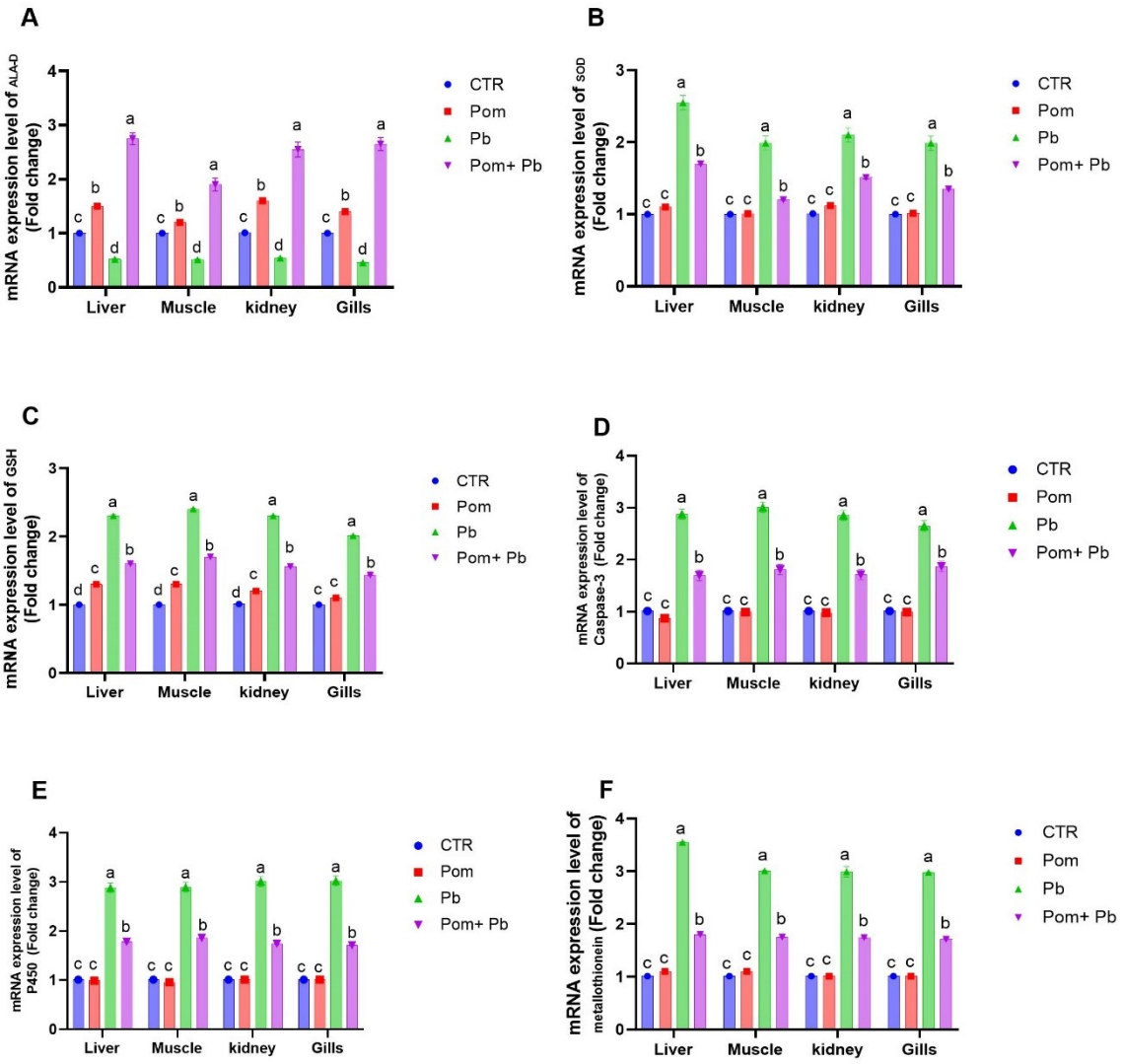

Fig. 2. ALAD, SOD, GSH, Caspase-3, Cytochrome P450, and Metallothionein mRNA gene expression in the liver, muscle, kidney, and gills of Nile tilapia fish in the different experimental groups. (A) Amino levulinate dehydratase (ALAD). (B) Superoxide dismutase (SOD). (C) Reduced glutathione (GSH). (D) Caspase-3. (E) Cytochrome P450. (F) Metallothionein. CTR = (control group), Pom = (fish group received pomegranate pulp supplemented diet 15%), Pb = (fish group received 24.4 mg lead acetate/L water), and Pom+ Pb = (fish group received pomegranate pulp supplemented diet 15% and 24.4 mg lead acetate/L water). The data are shown as the mean ± SE. The data were analyzed using a one-way ANOVA followed by the Tukey post-hoc multiple comparisons test. The values with different superscripts (a–d) in the same row indicate significant differences ($p < 0.05$).

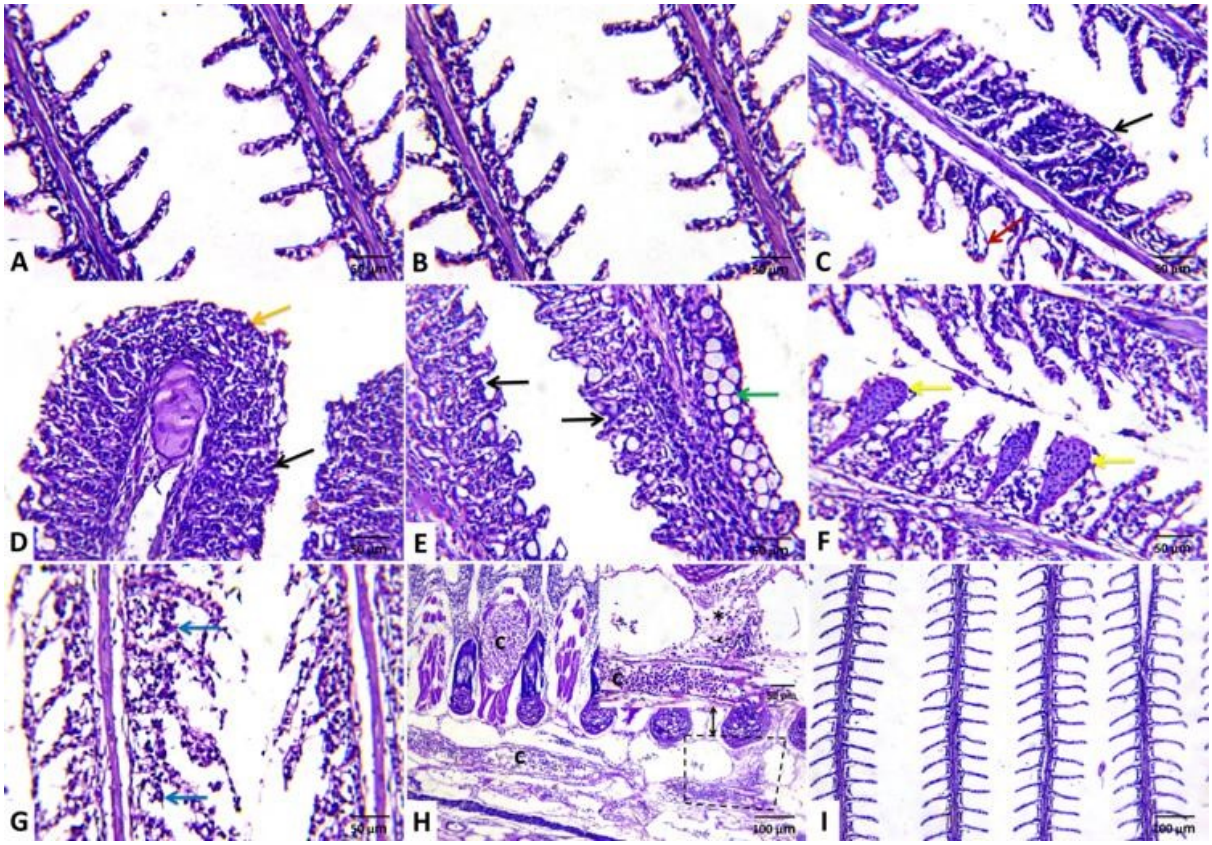

Fig. 3. Representative photomicrographs of Nile tilapia gills (HE, × 400). A, Control and B, BOM-treated fish, respectively, showing normal histoarchitecture of the of primary and secondary lamellae. C–H, Pb- intoxicated fish showing epithelial lifting (red arrow), epithelial hyperplasia of the primary lamellae with complete (black arrows) lamellar fusion, filamentous clubbing (orange arrow), goblet cell hyperplasia (green arrow), telangectiasis (yellow arrows), lamellar epithelial cells necrosis and desquamation (blue arrows), congestion(C), edema (*), and inflammatory cells infiltrations (dashed arrow) in the gill arch. I, BOM+ Pb -intoxicated fish showing marked improvement of the gills' histoarchitecture.

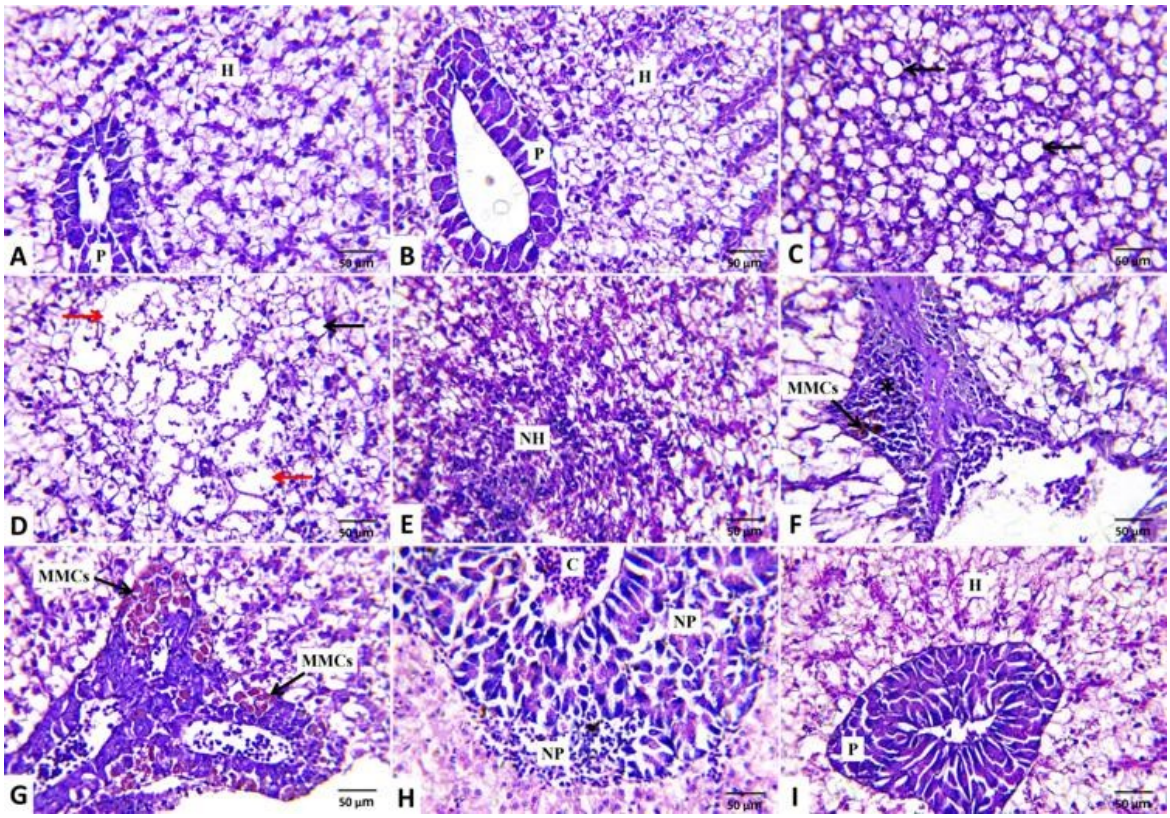

Fig. 4. Hepatopancreas in Nile tilapia representative photomicrographs (HE, × 400). A, Control and Fish treated with BOM in B exhibit normal hepatocyte histoarchitecture (H) and intact pancreatic acini (P) with evident eosinophilic zymogen granules. Pb-intoxicated fish in C-H exhibit perivascular mononuclear cell infiltrations (*), vascular congestion (C), ruptured fat-bearing cells forming fat cysts (red arrows), necrosis of the hepatocytes (NH) associated with mononuclear cells infiltration, marked activation of the melanomacrophage center (MMCs), and hepatocytic vacuolation of fatty type (black arrows) linked to pyknotic and eccentric nuclei. I, BOM+ Pb-intoxicated fish exhibiting a discernible improvement in the architecture of the pancreas (P) and liver (H).

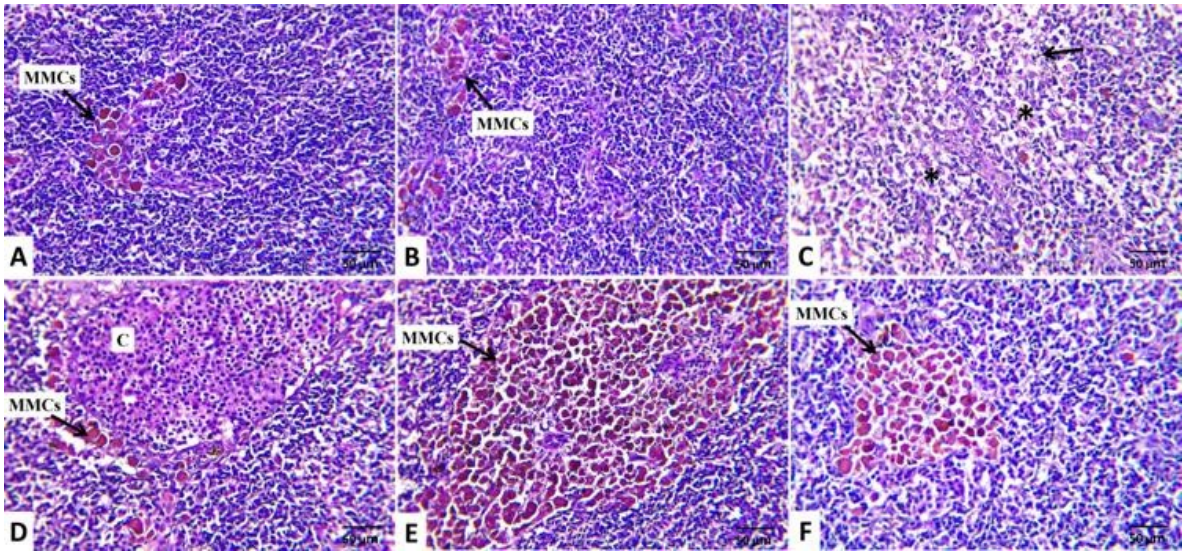

Fig. 5. Representative photomicrographs of Nile tilapia kidneys (HE, × 400). A, Control, and B, BOM-treated fish showed normal renal tubules, glomeruli, and interstitial connective tissues. c–e Pb- intoxicated fish showing tubular epithelial attenuation and necrosis (red arrow) with narrowing, glomerular atrophy with markedly dilated Bowman's space (yellow arrow), and tubulointerstitial nephritis with mononuclear cell infiltrations (*), interstitial hemorrhage(H), and hyperactivation of the melanmicrophage centers (MMCs). F, BOM+ Pb - intoxicated fish showing marked improvement of the renal tissue structure with moderate tubulointerstitial nephritis, moderate mononuclear cell infiltrations (*), and moderate MMCs activation.

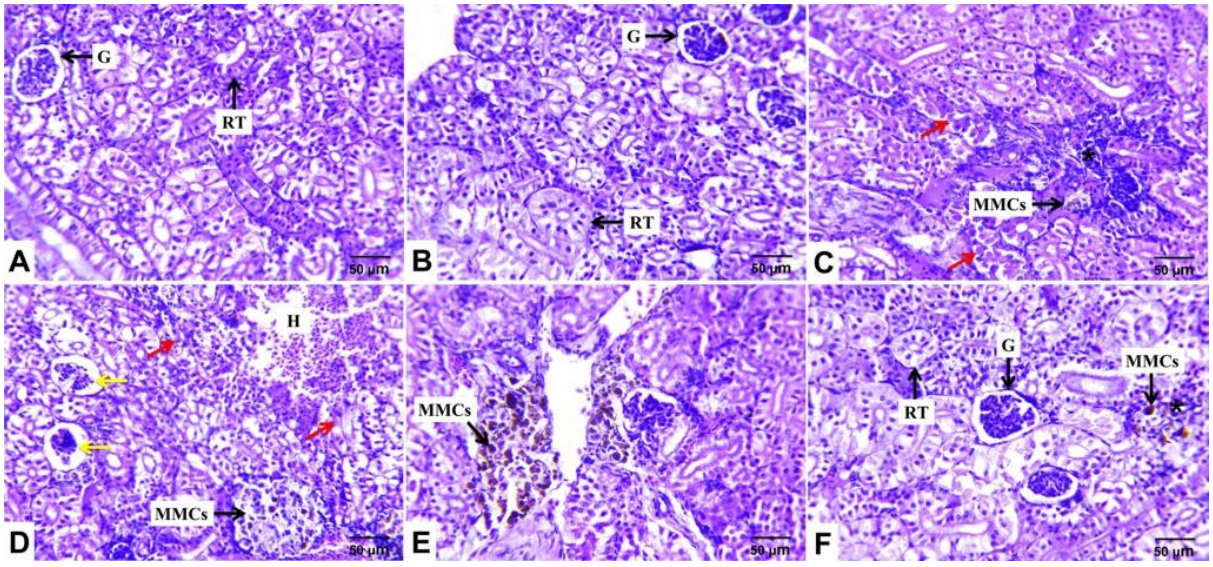

Fig. 6. Spleens of Nile tilapia, representative photomicrographs (HE, × 400). Normal histoarchitecture of the white pulp with closely packed basophilic cells, normal eosinophilic cells of the red pulp, and normal activity of melanomacrophage centers (MMCs) were seen in A, CTR, and B, Pom dietary supplemented fish, respectively. C–E, Pb-treated fish displaying hyperactivation of the Melano macrophage center (MMCs), vascular congestion (C), and lymphocyte depletion (*) and necrosis (arrow). F, Fish treated with Pom+ Pb had nearly complete repair of the splenic histoarchitecture along with a moderate level of MMC activation.

References

1. Béné, C. and S. Heck, *Fish and food security in Africa*. 2005.
2. Mapenzi, L.L. and A.J. Mmochi, Role of salinity on growth performance of Oreochromis niloticus♀ and Oreochromis urolepis urolepis♂ hybrids. *Journal of Aquaculture Research and Development*, **7**(6), 1000431 (2016).
3. Annaç, E., Uçkun, M., Özkaya, A., Yoloğlu, E., Pekmez, H., Bulmuş, Ö. and Aydın, A. The protective effects of pomegranate juice on lead acetate- induced neurotoxicity in the male rat: A histomorphometric and biochemical study. *Journal of Food Biochemistry*, **46**(4), e13881(2022).
4. Ezzat, N., Al-Hawary, I., Elshafey, A., Althobaiti, N. and Elbialy, Z. I. Effect of Seasonal Changes in Heavy Metals on the Histomorphology of the Liver and Gills of Nile Tilapia (Oreochromis niloticus L.) in Burullus Lake, Egypt. *Egyptian Journal of Veterinary Sciences*, **55**(6), 1705-1716(2024).
5. Järup, L., Hazards of heavy metal contamination. *British Medical Bulletin*, **68**(1), 167-182 (2003).
6. Wu, X., Cobbina, S. J., Mao, G., Xu, H., Zhang, Z.. and Yang, L., A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. *Environmental Science and Pollution Research,* **23**, 8244-8259 (2016).
7. Zeitoun, M.M. and Mehana, E. Impact of water pollution with heavy metals on fish health: *overview and updates. Global Veterinaria,* **12**(2), 219-231, (2014).
8. Kaur, G., Saroch, J. D., Shrivastav, R. and Qureshi, T. A., Correlative remedial effects of Spirulina and vitamin E on the histoarchitecture of liver and kidney of mercuric chloride challenged catfish Clarias gariepinus. *Journal of Chemical, Biological and Physical Sciences* (JCBPS), **2**(4), 2448 (2012).
9. Cheng, H. and Y. Hu, *Lead (Pb)* isotopic fingerprinting and its applications in lead pollution studies in China: a review. *Environmental Pollution,* **158**(5), 1134-114 (2010).
10. Ara, A. and J.A. Usmani, Lead toxicity: a review. *Interdisciplinary Toxicology,* **8**(2), 55-64 (2015).
11. Alkahemal-Balawi, H. F., Ahmad, Z., Al-Akel, A. S., Al-Misned, F., Suliman, E. A. M. and Al-Ghanim, K. A. Toxicity bioassay of lead acetate and effects of its sub-lethal exposure on growth, haematological parameters and reproduction in Clarias gariepinus. *African Journal of Biotechnology*, **10**(53), 11039 (2011).
12. Selvam, R. Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. *Urological Research*, **30**, 35-47 (2002).

13. Flora, S., Mittal, M. and Mehta, A. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian Journal of Medical Research,* **128**(4), 501-523 (2008).

14. Badrey, A. A., Osman, A., S Farrag, M., MM Toutou, M. and A Moustafa, M., Influences of diets supplemented with pomegranate peel on haematology, blood biochemistry and immune status in monosex Nile tilapia, Oreochromis niloticus. *Egyptian Journal of Aquatic Biology and Fisheries,* **23**(2), 133-144 (2019).

15. Orzua, M. C., Mussatto, S. I., Contreras-Esquivel, J. C., Rodriguez, R., De La Garza, H., Teixeira, J. A. and Aguilar, C. N. Exploitation of agro industrial wastes as immobilization carrier for solid-state fermentation. *Industrial Crops and Products,* **30**(1), 24-27 (2009).

16. Badawi, M.E. and Gomaa, A.M. Influence of diets supplemented with pomegranate peel extract on performance in Oreochromus niloticus. *Japanese Journal of Veterinary Research,* **64**(Supplement 2), S87-S94 (2016).

17. Türkyılmaz, M., Tağı, Ş., Dereli, U. and Özkan, M., Effects of various pressing programs and yields on the antioxidant activity, antimicrobial activity, phenolic content and colour of pomegranate juices. *Food Chemistry,* **138**(2-3), 1810-1818 (2013).

18. Kaderides, K., Goula, A.M. and Adamopoulos, K.G. A process for turning pomegranate peels into a valuable food ingredient using ultrasound-assisted extraction and encapsulation. *Innovative Food Science & Emerging Technologies,* **31**, 204-215 (2015).

19. Jurenka, J. Therapeutic applications of pomegranate (Punica granatum L.): a review. *Alternative Medicine Review*, **13**(2),128-144(2008).

20. Saeed, M., Naveed, M., BiBi, J., Kamboh, A. A., Arain, M. A., Shah, Q. A. and Dhama, K. The promising pharmacological effects and therapeutic/medicinal applications of punica granatum L.(Pomegranate) as a functional food in humans and animals. *Recent Patents on Inflammation & Allergy Drug Discovery,* **12**(1), 24-38 (2018).

21. Bianchi, M., *et al., FAO:* the state of world fisheries and aquaculture. *Food and Agriculture Organization of the United Nations: Rome, Italy,* 1-230 (2014).

22. Abdel- Hay, A. H. M., Emam, W., Omar, A. A., Eltras, W. F. and Mohamed, R. A., The effects of rearing water depths and feed types on the growth performance of African catfish (Clarias gariepinus). *Aquaculture Research,* **51**(2), 616-622 (2020).

23. Dawood, M. A., Shukry, M., Zayed, M. M., Omar, A. A. E. D., Zaineldin, A. I. and El-Basuini, M. F. Digestive enzymes, immunity and oxidative status of Nile tilapia (Oreochromis niloticus) reared in intensive conditions. *Slov. Vet. Res.,* **56**(Suppl 22), 99-108 (2019).

24. Zhang, L., Xu, E. G., Li, Y., Liu, H., Vidal-Dorsch, D. E. and Giesy, J. P. Ecological risks posed by ammonia nitrogen (AN) and un-ionized ammonia (NH3) in seven major river systems of China. *Chemosphere,* **202**, 136-144 (2018).

25. El Euony, O. I., Elblehi, S. S., Abdel-Latif, H. M., Abdel-Daim, M. M. and El-Sayed, Y. S., Modulatory role of dietary Thymus vulgaris essential oil and Bacillus subtilis against thiamethoxam-induced hepatorenal damage, oxidative stress, and immunotoxicity in African catfish (Clarias garipenus). *Environmental Science and Pollution Research,* **27**, 23108-23128 (2020).

26. Ang, C. Comparison of broiler tissues for oxidative changes after cooking and refrigerated storage. *Journal of Food Science,* **53**(4),1072-1075 (1988).

27. Vitošević, B., Samardžić, S., Antonijević, V. and Jakovljević, V. Heavy metals in some imported food products and their potential toxic implications. *Medicus,* **8**(2), 62-66 (2007).

28. Livak, K.J. and Schmittgen,T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods,* **25**(4), 402-408 (2001).

29. Kranz, H. and Gercken,J. Effects of sublethal concentrations of potassium dichromate on the occurrence of splenic melano- macrophage centres in juvenile plaice, Pleuronectes platessa, L. *Journal of Fish Biology,* **31**, 75-80 (1987).

30. Diab, M.A., Effect of Aqueous Solutions of Pomegranate Peel on Some Heavy Metals in Grey Mullet (Mugilcephalus Linnaeus, 1758). *مجلة دراسات وبحوث التربية النوعية* **1**(1) **,** (2019).

31. Kahramanoglu, I. and Usanmaz,S. *Pomegranate production and marketing*. 2016: *CRC Press.*

32. Abozahra, N. A., Albalawi, A., Althobaiti, N., Mohamed, R. and Diab, A. Effects of Pomegranate (Punica granatum) Peel Methanolic Extract Dietary Supplementation on Oreochromis Niloticus Performance, Blood Health, Intestine Morphometry and Immunity. *Egyptian Journal of Veterinary Sciences,* **54**(7), 221-236 (2023).

33. Scott, G. R., Sloman, K. A., Rouleau, C. and Wood, C. M. Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (Oncorhynchus mykiss). *Journal of Experimental Biology,* **206**(11), 1779-1790 (2003).

34. Alinnor, I. Assessment of elemental contaminants in water and fish samples from Aba River. *Environmental Monitoring and Assessment,* **102**, 15-25 (2005).

35. Farah, M. A., Ateeq, B., Ali, M. N. and Ahmad, W. Evaluation of genotoxicity of PCP and 2, 4-D by micronucleus test in freshwater fish Channa punctatus. *Ecotoxicology and Environmental Safety,* **54**(1), 25-29 (2003).

36. Paul, S., Mandal, A., Bhattacharjee, P., Chakraborty, S., Paul, R. and Mukhopadhyay, B. K. Evaluation of water quality and toxicity after exposure of lead nitrate in fresh water fish, major source of water pollution. *The Egyptian Journal of Aquatic Research,* **45**(4), 345-351 (2019).

37. Bellahsen, N., Varga, G., Halyag, N., Kertész, S., Tombácz, E. and Hodúr, C., Pomegranate peel as a new low-cost adsorbent for ammonium removal. *International Journal of Environmental Science and Technology,* **18**, 711-722 (2021).

38. El Barnossi, A., Moussaid, F. and Housseini, A.I. Tangerine, banana and pomegranate peels valorisation for sustainable environment: A review. *Biotechnology Reports,* **29**, e00574 (2021).

39. El-Ashtoukhy, E.-S., Amin, N. and Abdelwahab, O. Removal of lead (II) and copper (II) from aqueous solution using pomegranate peel as a new adsorbent. *Desalination,* **223**(1-3), 162-173 (2008).

40. Li, Y., Guo, C., Yang, J., Wei, J., Xu, J. and Cheng, S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry,* **96**(2), 254-260 (2006).

41. Singh, G., Maurya, S., De Lampasona, M. P. and Catalan, C. Chemical constituents, antifungal and antioxidative potential of Foeniculum vulgare volatile oil and its acetone extract. *Food Control,* **17**(9), 745-752 (2006).

42. Kandylis, P. and E. Kokkinomagoulos, Food applications and potential health benefits of pomegranate and its derivatives. *Foods,* **9**(2), 122 (2020).

43. Ay, Ç. Ö., Özcan, A. S., Erdoğan, Y. and Özcan, A. Characterization of Punica granatum L. peels and quantitatively determination of its biosorption behavior towards lead (II) ions and Acid Blue 40. *Colloids and Surfaces B: Biointerfaces,* **100**, 197-204 (2012).

44. Ben-Ali, S., Jaouali, I., Souissi-Najar, S. and Ouederni, A. Characterization and adsorption capacity of raw pomegranate peel biosorbent for copper removal. *Journal of Cleaner Production,* **142**, 3809-3821 (2017).

45. De Smet, H. and Blust, R. Stress responses and changes in protein metabolism in carp Cyprinus carpio during cadmium exposure. *Ecotoxicology and Environmental Safety,* **48**(3), 255-262 (2001).

46. Van Dyk, J.C., Pieterse,G. and Van Vuren,J. Histological changes in the liver of Oreochromis mossambicus (Cichlidae) after exposure to cadmium and zinc. *Ecotoxicology and Environmental Safety,* **66**(3), 432-440 (2007).

47. Mela, M., Randi, M. A. F., Ventura, D. F., Carvalho, C. E. V., Pelletier, E. and Ribeiro, C. O. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish Hoplias malabaricus. *Ecotoxicology and Environmental Safety,* **68**(3), 426-435 (2007).

48. Heath, A. Water pollution and physiology. *CRC press Inc., Boca Raton,* (1987).

49. Nordberg, G.F., Nordberg, M., Piscator, M. and Vesterberg, O. Separation of two forms of rabbit metallothionein by isoelectric focusing. *Biochemical Journal,* **126**(3), 491-498 (1972).

50. Gupta, P. and Srivastava, N. Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish Channa punctatus(Bloch). *Journal of Environmental Biology,* **27**(2), 211-215 (2006).

51. Kaoud, H. and El-Dahshan, A. Bioaccumulation and histopathological alterations of the heavy metals in Oreochromis niloticus fish. *Nature and Science,* **8**(4), 147-156 (2010).

52. Lock, R. and Bonga, S.W. Toxicants and osmoregulation in fish. *Netherlands Journal of Zoology,* **42**(2-3), 478-493 (1991).

53. Khan, H. A., Malabika Sikdar-Bar, M. S. B., Kamlesh, B., Wani, A. A. and Pervaiz, P. A. Lead nitrate induced histopathological changes in the gills of the African Clarias batrachus. *Journal of Applied Sciences Research,* **7**(7), 1081-1086 (2011).

54. Mohammed, F. Histopathological studies on Tilapia zilli and Solea vulgaris from lake Quran, Egypt. *World J. Fish Mar. Sci,* **1**, 29-39 (2009).

55. DE, H. Integrative histopathological effects of environmental stressor on j fishes. *in American Fisheries Society Symposium* (1990).

56. Agah, H., Leermakers, M., Elskens, M., Fatemi, S. M. R., & Baeyens, W. Accumulation of trace metals in the muscle and liver tissues of five fish species from the Persian Gulf. *Environmental Monitoring and Assessment,* **157**, 499-514 (2009).

57. Roy, S. and Bhattacharya,S. Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of Channa punctatus. *Ecotoxicology and Environmental Safety,* **65**(2), 218-229 (2006).

58. Authman, M., Oreochromis niloticus as a biomonitor of heavy metal pollution with emphasis on potential risk and relation to some biological aspects. *Global Veterenaria,* **2** (3), 104–109 (2008).

59. Liao, C. Y., Fu, J. J., Shi, J. B., Zhou, Q. F., Yuan, C. G., & Jiang, G. B. Methylmercury accumulation, histopathology effects, and cholinesterase activity alterations in medaka (Oryzias latipes) following sublethal exposure to methylmercury chloride. *Environmental Toxicology and Pharmacology,* **22**(2), 225-233 (2006).

60. Harikrishnan, R., Kim, J. S., Kim, M. C., Balasundaram, C., & Heo, M. S. Pomegranate enriched diet enhances the hematology, innate immune

response, and disease resistance in olive flounder against Philasterides dicentrarchi. *Veterinary Parasitology,* **187**(1-2), 147-156 (2012).

61. Yousefi, M., Hoseini, S. M., Kulikov, E. V., Babichev, N. V., Bolshakova, M. V., Shopinskaya, M. I. and Zharov, A. N., Effects of dietary pomegranate peel supplementation on growth performance and biochemical responses of common carp, Cyprinus carpio, to chronic crowding stress. *Aquaculture Reports,* **30**, 101532 (2023).

التخفيف من الآثار الضارة لأسيتات الرصاص على صحة البلطي النيلي من خلال مكملات قشر الرمان: النمو ، والتشكل ، وجودة اللحم ، والاستجابات المضادة للأكسدة ، والتعبير الجيني ، وموت الخلايا المبرمج.

سحر حمد النجار [1]، رضا عبد الله [2]، عبد الله صلاح [3، 4]، أميرة عمر [5]، مصطفى شكري [6]، الدسوقي ناصف [7]، دعاء درغام [8]، مصطفى المعداوي [9]، سمر البلهي [10].

[1] قسم تنمية الثروة الحيوانية، كلية الطب البيطري، جامعة كفر الشيخ، كفر الشيخ، مصر. sahr_hamdy@vet.kfs.edu.eg

[2] قسم الرقابة علي الأغذية ، كلية الطب البيطري ، جامعة كفر الشيخ ، كفر الشيخ 33516 ، مصر ؛ reda_abdallah@vet.kfs.edu.eg

[3] قسم الاستزراع المائي، كلية علوم الأحياء المائية والسمكية، جامعة كفر الشيخ، كفر الشيخ، مصر.

[4] معهد الاستزراع المائي، جامعة ستيرلنغ، ستيرلنغ، المملكة المتحدة

[5] قسم أمراض الأسماك ورعايتها ، كلية الطب البيطري جامعة كفر الشيخ ، كفر الشيخ 33516 ، مصر

[6] قسم علم وظائف الأعضاء، كلية الطب البيطري، جامعة كفر الشيخ، مصر.

[7] قسم التغذية والتغذية الإكلينيكيه، كلية الطب البيطري، جامعة كفر الشيخ، 33516، مصر

[8] قسم الكيمياء الحيوية، كلية الطب البيطري، جامعة كفر الشيخ، مصر.

[9] قسم الطب الشرعي والسموم، كلية الطب البيطري، جامعة كفر الشيخ، مصر.

[10] قسم باثولوجيا الأمراض ، كلية الطب البيطري ، جامعة الإسكندرية ، إدفينا 22758 ، مصر.

المستخلص

يعد التلوث بالرصاص (Pb) ، الناجم عن الأنشطة البشرية ، مشكلة سائدة في البيئات المائية ويشكل مخاطر كبيرة على صحة الأسماك والبشر. الأسماك ، على وجه الخصوص ، معرضة بشدة لتراكم الرصاص ، مما يعوق نموها ورفاهها العام. من المتوقع أن تفحص هذه التجربة الآثار الضارة لسمية أسيتات الرصاص على جوانب مختلفة من صحة البلطي النيلي ، بما في ذلك العوامل الدموية والكيميائية الحيوية ، والإجهاد التأكسدي ، والاستجابة المناعية ، وجودة المياه ، جودة عضلات الأسماك ، والتعبير الجيني. بحثت الدراسة أيضا في الآثار المخففة المحتملة للمكملات الغذائية مع قشر الرمان. تم تقسيم مائة وعشرين من البلطي النيلي الصحي من نفس الأحجام إلى أربع مجموعات تجريبية: مجموعة ضابطة، ومجموعة مكملة بلب الرمان، ومجموعة معرضة لأسيتات الرصاص، ومجموعة معرضة لكل من قشر الرمان وخلات الرصاص. تم تغذية الأسماك بوجباتها الغذائية لمدة ستة أسابيع ، وتم تقييم معايير مختلفة .

كشفت النتائج أن التعرض لأسيتات الرصاص أعاق نمو الأسماك بشكل كبير وأدى إلى زيادة المؤشرات الحيوية للإجهاد ، مما أدى إلى تلف الكبد والفشل الكلوي.

علاوة على ذلك ، أثرت أسيتات الرصاص سلبا على المعلمات المناعية وتسببت في ارتفاع مستويات الإجهاد التأكسدي في الكبد والكلى. ومع ذلك ، فإن مكملات قشر الرمان خففت بشكل فعال من هذه الآثار الضارة من خلال تعزيز مضادات الأكسدة والأنشطة المناعية.

وأظهرت الدراسة أيضا أن مكملات الرمان قللت من تلف العضلات وحسنت جودة لحوم الأسماك.

بالإضافة إلى ذلك ، فقد أثر على التعبير الجيني المرتبط بنشاط مضادات الأكسدة ، وموت الخلايا المبرمج ، وإزالة السموم في مختلف أعضاء البلطي النيل. في الختام ، كان لأسيتات الرصاص عواقب ضارة على أداء البلطي النيلي ، لكن المكملات الغذائية مع قشر الرمان أثبتت أنها استراتيجية فعالة للتخفيف من هذه الآثار. يعزز قشر الرمان الدفاعات المضادة للأكسدة ، ويعزز الاستجابة المناعية ، ويحسن الصحة العامة ، وبالتالي يقلل من الآثار الضارة للتعرض للرصاص.

الكلمات الدالة: قشر الرمان, سمية الرصاص, جودة اللحم, علامات مضادة للأكسدة, أوريوكروميس نيلوتيكوس.