Egyptian Journal of Veterinary Sciences

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

Quillaja saponaria Improved Growth Performance, Hemato-biochemical Profile and Protects Nile Tilapia *(Oreochromis niloticus)* Against Pb and Cd-Induced Oxidative and Inflammatory Tissues Damage

Samar Tawfik [1], Malik M. Khalafalla[1], Mohamed M. Zayed[1], Salma M. Zeid[2] and Radi A. Mohamed[1]

[1] *Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafr El-sheikh 33516, Egypt.*

[2] *Department of Oceanography, Faculty of Science, Alexandria University, Alexandria 21568, Egypt.*

Abstract

THIS STUDY AIMED to estimate the diminishing effect of Quillaja saponaria (QS) on Nile tilapia exposed to heavy metals and its effect on growth performance, hemato-biochemical profile, oxidative response and tissue damage. Fish (N=90, average weight: 61.74 ± 4.72 g) were randomly distributed in triplicates at a rate of 6 fish per 60 L aquarium. Fish exposed to cadmium (Cd) and lead (Pb) mixture with 1.31 mg/L and 58.4 µg/L, respectively and received QS with 150, 300, 500 mg/kg fish feed. Pd and Cd significantly decreased growth performance and feed utilization. Metals deteriorated haemato-biochemical parameters by decreased Hb, HTC and RBCs, increasing WBCs, increasing the levels of ALT, AST, ALP, bilirubin, urea, triglycerides, glucose and total lipids and decreasing total protein, globulin and HDL. Observed increase of MDA levels and decrease SOD, GSH and ChE% activities were recorded in metals- exposed fish. Challenge with metals revealed up-regular effect of liver CYP19 gene expression. QS enhanced fish growth performance while exposure to metals. QS restored the normal haemato-biochemical indices compared to the control. QS reduced the effect of metals on oxidative status enzymes (decreased MDA values and increased SOD, GSH and ChE%) activities. QS achieved the balance of cell proliferation and apoptosis of CYP19 and GPx gene expressions. In conclusion, QS has diminished effect against Cd and Pb negative impacts on fish. QS concentrations 150, 300 and 500 mg/kg fish feed enhanced growth performance, haemato-biochemical indices, hindered the oxidative stress and achieved cell balance of proliferation and apoptosis.

Keywords: Cadmium, *Quillaja saponaria*, *Oreochromis niloticus*, heavy metals, oxidative status.

Introduction

Heavy metals (HMs) are widespread contaminants of water affecting aquatic ecosystem [1, 2]. Although they are common in the nature, the anthropogenic sources as industries, mining, agriculture, domestic wastes and sewage increase their levels [3, 4]. These activities release a big quantities of HMs into the environment causing metal pollution [5]. HMs are non-biodegradable and persistent substances in the environment [6, 7].The aquatic environment is considered a habitat for aquatic species and a reservoir of potential persistent elements [8]. Although different physico-chemical and biological mechanisms found in the aquatic ecosystems fit to remove or decrease adverse toxic substances' effects, Bioavailability of metal ions inside the living cell may induce alterations in development, growth, reproduction, behaviour of organisms and/or death of injured cells [9, 10]. Cadmium (Cd) and lead (Pb) are a significant group of environmentally risky metals if present [11]. They have a worldwide specific concern [12]. Cd and Pb can incorporate into the food chain through water or food consumption to levels that affect their physiological state and cause great threats to their beings in the food chain [13, 7]. Being fish one of desired human food, it becomes an indirect source of HMs arriving the human body

*Corresponding author: Radi A. Mohamed, E-mail: r.mohamed.vet@gmail.com, Tel.: +201004255067
(Received 14 March 2024, accepted 13 August 2024)
DOI: 10.21608/EJVS.2024.277041.1916

[14]. Accumulation of metals in fish organs is subjective to some factors as surrounded environmental conditions (Dissolved Oxygen, temperature, salinity, pH etc.), biological factors (species, sex, size and age) and feeding sources [15, 16, 14,17]. Effects differ according to the metal species and water mineralization degree [18]. Metal toxicity can affect blood composition processes and injures vital tissues [19]. Influences on fish may include growth inhibition, reduction of reproduction rates, hampering antioxidant capacity, and boosting sensitivity to pathogens [20, 21]. The problem occurs through the production of reactive oxygen species because of accumulation of metals causing oxidative stress and carcinogenesis in addition to behavioral, biochemical, and molecular changes [22].

As good and safe fish quality demand increases, several plant extracts have been broadly involved in fish diet for enhancement of fish growth performance and improvement their diseases resistance [23]. One of the widespread plant extracts is saponin. Saponin is found in diverse of plants which have hydrophobic aglycone. The aglycone may be a steroid or triterpenoid in nature on the way to which one or more sugar chains are involved [24]. Triterpenoid saponins are found in the soapbark tree, *Quillaja saponaria,* which is a conventional medicinal tree native to the Andes region - Colombia, and naturally formed glycosides. *Quillaja saponaria* (QS) is mostly formed by plants and existed in bacteria as well some lower marine organisms [25, 26]. It possesses strong biological activity partly owing to the presence of Quillaic acid [27]. In the past, saponins were mainly considered as a detergent source [28, 29, 30]. Nowadays, they are approved in human diet due to their widespread existence in food additives [25]. Saponins are studied for their role as food constituents in 187 countries [29]. QS is known to its uses as antibiotic and antimicrobial activities [31]. They are one known constituents in plant having a wide biological applications on organisms [32]. QS has a wide application in aquaculture. Extensive research has been approved the effective role of QS as a natural growth promoter when added into the aquafeeds [33,27, 24,34]. It helps enhancing the fish immune-defense mechanism [35, 34], reducing fish mortality [31], protecting against oxidative stress [35]. Elkaradawy et al., [26] revealed the ability of QS in improving water quality and the fish performance. Dietary QS could potentially replace hazardous synthetic androgens in producing all-male populations or reducing female's fertility if fed to tilapia at an early age [25]. Some studies recorded enhancement of antioxidant enzymes of fish received the diet supplemented with QS [26, 34]. There is no available data on the impact of dietary supplementation of QS against HMs induced toxicity in fish.

The modern aquaculture practices are mainly depending on the intensive and super-intensive farming systems for aquatic species [36]. Worldwide, the aquaculture faces the challenge to optimize its production through sustainable practices [37]. Incorporation metal ions into farmed fish to that level which altering their physiological functions affect the health of consumers and threats the fish production industry [38]. Nile tilapia is one of the most economically important fish species cultivated in the world [39]. It has the ability to fed on different types of foods, survive in stressed environments and has strong immune system [40]. It is considered a sentinel species of environmental quality [39] and commonly used as an excellent bio-indicator for assessment of HMs contamination and their risks for consumers [41, 19,42,35]. The aim of this study was to evaluate the QS effects on growth performance, haematology, antioxidant capacity, biochemical profile, oxidative status and the health of gills, brain, liver and spleen of Nile tilapia exposed to mixture of HMs.

Material and Methods

Ethical approval

The protocol of this study is reviewed by the institutional committee for the use of aquatic animals in research, faculty of Aquatic and Fisheries Sciences, University of Kafrelsheikh.

Preparation of synthetic heavy metals

Synthetic mixtures of Cd and Pb in the present study, prepared as previously recorded levels measured by Abdel-Tawwab et al. [43] in drainage polluted water (1.31 mg/L and 58.4 µg/L for Cd and Pb, respectively). Lead nitrate Pb $(NO_3)_2$ and cadmium chloride ($CdCl_2.2.5H_2O$) (Sigma-Aldrich Chemicals, USA) used to prepare stock solutions. Desirable concentrations were first dissolved in distilled water. Throughout the experimental period, pre-prepared solutions were added during the aquarium water exchange each 2 days from freshly prepared stock solutions for prevention of concentration changes caused by adsorption and evaporation processes [44]. All the other chemicals were of analytical grade.

Diets preparation, fish management, and study design

Nile tilapia (*Oreochromis niloticus*) was obtained from private fish farm (Kafr El-Sheikh governorate, Egypt). Fish were kept for adaptation in indoor tanks for two weeks for the laboratory condition (12 – 12h light-dark photoperiod cycle). Fish (N=90, average

weight: 61.74 ± 4.72 g) were randomly distributed in triplicates at a rate of 6 fish per 60 L aquarium. The tested experimental groups were (1) C-G; control group, fish fed on the basal diet, (2) HM-mix.; pre-prepared heavy metals' mixture (mixture Cd and Pb solution) added into the water and fish received a basal diet, (3) QS_{500}; fish were fed on the basal diet containing *Quillaja Saponaria* (QS) extract (ENVIRO QS®- Delacon Biotechnik GmbH, Austria) with concentration 500 mg/kg fish diet reared in water containing HMs, (4) QS_{300}: fish were fed on the basal diet containing 300 mg QS /kg fish diet reared in pre-prepared HMs containing water, (5) QS_{150}: fish were fed on the basal diet containing 150 mg QS /kg fish diet reared in pre-prepared HMs containing water. QS powder was gently added to the basal diet after the diet components were minced to obtain a homogenous mixture and stored at -20 C in opaque plastic containers. The basal diet was 30/6, protein/lipids ratio, with 18.73 MJ kg1 gross energy. Each aquarium was supplied with compressed air via air-stones (using aquarium's air pump) and a mechanical filter (to collect the fish waste). Fish were fed diets twice daily at 8:00 am and 14:00 pm for 40 days. Daily fish mortalities were recorded for each aquarium. Water parameters were recorded daily in each aquarium. Total ammonia nitrogen (TAN) was measured using a portable colorimeter (Milwaukee-Mi 405), Dissolved oxygen (DO) using OxyGuard handy Polaris dissolved oxygen, pH and temperature using pH meter (HANNA- HI98191- PH meter). Water quality was under the natural conditions (temperature was 26.95 ± 1.7 °C, pH 7.63 ± 0.51, TAN 0.04 ± 0.01 mg/L and DO 5.32 ± 1.62 mg/L) and all parameters were represented as mean ± SEM) [45].

Fish growth performance and feed utilization efficiency

To calculate Fish feed utilization and growth performance, at the end of the 40-days trial period, the fish were collected and anesthetized using clove oil (50 ml/L of water, Merck, Germany). Each fish was weighed separately using digital balance to get the final weight. Growth evaluation variables were measured as described by Abozahra et al. [46] and Ghalwash et al. [45] as follows: weight gain (WG) = (W_1-W_0), specific growth rate (SGR, % body weight/day) = $100 \times [(\ln W_1-\ln W_0)/t]$. Feed conversion ratio (FCR) was calculated as FI (g)/WG (g). Fish survival (%) = 100 × [final number of fish/ initial number of fish]. Where W_0 is the initial weight, W1 is the final weight (g), L is the final fish length and t is the experimental period in days.

Blood collection and serum separation

At the end of the trial, fish feeding was stopped 24 h proximately prior to blood sampling. From the fish caudal vein, blood samples were taken using heparinized syringe containing EDTA (Ethylenediamine tetra acetic acid) as an anticoagulant and gathered in vacuum tubes (3 replicate/aquarium) [47]. For blood serum collection, the assembled blood was collected without anticoagulants and centrifuged at the room temperature at 300 x g for 15 min and the supernatant serum was stored in plastic Eppendorf tubes at –20 ^{0}C [48].

Haematological analyses

Red blood cells (RBCs) count, white blood cell (WBCs) count, Haemoglobin content (Hb), Haematocrit (HTC), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) using an automatic blood cell counter (Exigo- Vet., Boule Medical AB Inc., Stockholm, Sweden) [49]. For the other leucocytes count check, from each blood test, two thin films of blood were regulated on previously dry, pre-cleaned microscope slide for staining using a modified Wright's stain. From each stained slide, a total of 100 cells were considered using ×100 oil immersion lens for investigation of lymphocytes, monocytes, Neutrophils and Eosinophils percentages (Exigo-Vet., Boule Medical AB Inc., Stockholm, Sweden) [50].

Serum biochemical analysis

Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities were measured colorimetrically at 540 nm wavelength [51]. Serum total proteins (TP) were estimated at the wavelength of 540 nm as illustrated by Doumas et al. [52] and albumin and bilirubin were measured at a wavelength 550 nm according to Dumas and Biggs, [53]. The globulin content was calculated by subtracting albumin from TP. Total lipids were evaluated as described by Zöllner, N. and Kirsch,[54]. Serum triglyceride (TG) were estimated using GPO- PAP and the total cholesterol (TC) using CHOD- PAP (commercial clinical kit) [55]. Creatinine was calorimetrically approximate according to Heinegård and Tiderström,[56]. Urea nitrogen and glucose were determined using Bio-Diagnostic Company kits (Bio-Diagnostic) and High density lipoprotein (HDL) was measured using commercial kits (Biodiagnostic Co., Egypt) [57].

Antioxidants status

Serum collected samples were used to estimate the oxidative stress assay. The activities of enzymes catalase (CAT) and superoxide dismutase (SOD),

malondialdehyde (MDA), were evaluated using ELISA kits (Inova Biotechnology, China) at the wavelength 450 nm using the microplate ELISA reader [58]. Glutathione (GSH) activity was determined colorimetrically, using commercial kits (Biodiagnostic Co., Cairo, Egypt), in accordance with the method described by Habig et al.[59]. Cholinesterase (ChE) activity were determined according to Brodeur et al.[60] .

Total RNA extraction, cDNA synthesis and real-time quantitative PCR assay

Total RNA was obtained from 50 mg of Nile tilapia livers with Trizol (iNtRON Biotechnology) following the manufacturer's instructions. The integrity of RNA was authorized by ethidium bromide stained 2% agarose gel electrophoresis. The concentration and purity of RNA were estimated using a Nanodrop BioDrop spectrophotometer (Biochrom Ltd, Cambridge CB23 6DW, UK) based on the A260/A280 nm ratio. Two µg of RNA sample were reverse transcribed using ABT 2X RT Mix cDNA synthesis kit according to manufacturer's Protocol. Gene expression profiling was performed in Rotor Gene-Q (Qiagen-Germany) using gene-specific primer sequences for the amplification of Cytochrome (*CYP19*) and glutathione peroxidase (*GPx*) as represented in Table 1. The amplification reaction was done using ABT 2X qPCR Mix (SYBR) kit. The reaction volume was 20 µl consisting of 10µL SYBR Green, 0.6 µL of forward and reverse specific primer, 1 µL of cDNA template, and nuclease-free water to make the final volume 20 µL. The PCR program was carried out with the following conditions: activation at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at the primer-specific temperature for 15 seconds, and extension at 72°C for 25 seconds. This was followed by a melt curve analysis to assess the specificity of amplification at 72°C to 95°C. All genes were tested in triplicates. CT values for each sample were determined and incorporated in "fold change" ($2^{-\Delta\Delta CT}$), calculation based on Livak and Schmittgen, [61], and mRNA expressions for each sample were normalized against the beta actin as a housekeeping gene.

Histopathological procedures

Specimens from liver, spleen brain and gills were taken immediately from the dissected fish. Samples were placed in a 10% prepared buffered formaldehyde solution for fixation. Specimens were fixed in paraffin wax then 3-4µm sections were cut by Leica Rotary Microtome (RM 2145, Leica Microsystems, Wetzlar, Germany). Slides were deparaffinized by running them in xylene to alcohol to water for histological staining. Each slide was stained using hematoxylin and eosin (H&E) stain according to Suvarna and Layton, [64]. for an investigation of the selected fish tissues and observation of the spreading, occurrence, and invasion of the inflammatory cells. The prepared tissue sections were examined using Leica ICC50W Image Analyzer, microscope at 10X, 20X and 40X magnification.

Statistical Analysis

After testing of data for normality and homogeneity and the normality was assessed using analysis of the residuals, a statistical analysis was performed using GraphPad Prism 6 (GraphPad Prism v6.0, San Diego, CA, USA). Data were evaluated for significant differences between the experimental groups with one- way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were confirmed at the 5% probability level. The obtained data were expressed as the mean ± standard error of the mean (SEM).

Results

Growth performance and feed utilization efficiency

Quillaja Saponaria dietary supplementations with different concentrations (150, 300, 500) revealed significant inhibition of the metals' negative effect on fish growth performance as recorded in Table 2. Challenge using Cd and Pb mixture effect negatively on WG, WG%, SGR and FCR. Fish groups received QS showed enhanced growth performance. In presence of metals, All QS groups displayed significant decrease of FCR ($P < 0.05$) to be close to the growth and feed utilization of the control group. Survival rate didn't display differences ($P > 0.05$).

Haematological parameters

Hemoglobin, HTC and RBCs are found significantly lower ($P<0.05$) in Nile tilapia exposed to metals' mixture in comparing with the control and QS dietary supplementations. WBCs counts increased significantly in fish exposed to HM challenge($P<0.05$), but supplementations with QS diminish the negative effect, especially QS_{150}. Lymphocytes, Neutrophils, Monocytes and Eosinophils displayed nonsignificant differences between groups ($p> 0.05$) as summarized in Table (3).

Serum biochemical profile

Exposure to Cd and Pb mixture caused significant deterioration in most of measured biochemical parameters (ALT, AST, total protein, globulin, ALP, urea, triglycerides and total lipids, HDL, glucose, bilirubin), ($P<0.05$) as illustrated in Table (4). Fish received QS supplementations in groups QS_{300} and QS_{150} achieved significant enhancement of serum

biochemical indices (P<0.05) in comparing with other groups.

Oxidative status

Quillaja Saponaria with its different concentrations accomplished significant enhancement of the fish oxidative status. The effect of Cd and Pb mixture and assimilation with QS supplementations on the oxidative status of Nile tilapia are shown in Figure (1). SOD and GSH showed significant decrease when fish exposed to metals but adding QS enhanced the harmful effect of metals on these enzymes (P<0.05). HMs increased values of MDA (P<0.05). QS ability diminishing HMs impacts mirror on decreasing MDA levels in QS_{500}, QS_{300} and QS_{150} groups. In addition, significant restoration (P<0.05) observed on ChE% activity when fish received QS feed additives.

Gene expression assay

Exposure of Nile tilapia to metals displayed exhibited up-regular effect of liver *CYP* gene expression (4.3-fold increase) but there wasn't significant change in liver *GPx* gene expression as represented in Figure 2. Treatment with QS supplementations showed different manners on tested genes. QS_{500} succeeded in up-regular liver *GPx* gene by 20-fold increase and down-regular *CYP* gene expression (0.3-fold change) in comparison with the C-G and HM-mix group.

Discussion

Any deviation of the water quality of fish pond may cause stress on cultured species and impact the fish health and its growth performance [7]. Our findings verify the thought that toxicological effect of metals on fish tissues are intervened across the stimulation of oxidative stress besides animal cells apoptosis. In the present study, exposure of Nile tilapia to a combination of Cd and Pb which accumulate and injure various organs, inhibited fish growth performance. There are no previous studies on the defeated effect of QS on fish exposed to HMs. This work showed significant depletion of Nile tilapia growth performance and feed utilization when exposed to metals. But fish fed with QS supplements (in presence of metals) showed restoration of their feed rate. QS with the different concentrations (150, 300, 500 mg/kg) were observed to have the ability to stimulate Nile tilapia growth, as described before by Levavi-sivan et al.[65] and Stadtlander [66]. Elkaradawy et al.[26] and Elkaradawy et al.[67] recorded improvement in the final body weight (FBW), WG, WGR of Nile tilapia received QS compared with the control. Our results agree with Merrifield and Guroy, [68] who fed striped catfish with different QS concentration supplements and found that WG, SGR and FCR significantly higher in groups fed QS. Although Deng et al.[34] informed that high doses of saponins may decreased growth values, QS_{500} group showed the highest growth performance. Also, Rezaei,[32] found SGR, WG and food efficiency of *Amatitlania nigrofasciata* were observed in male fed with 700 mg/kg QS and 300 mg/kg female. Improvement of growth performance of fish fed QS because of the amount of saponins which increase permeability of the cell membrane. It has been theorized that this could permit for enhance nutrient absorption [27, 68]. Exposing to chronic stress lowers the ability of the organism to trigger the defense mechanism, producing dysfunctional reaction, following growth inhibition. Hence, enhancing food absorption ability could improve FCR, and raise growth and feed efficiency [69].

Haematological indices are used more and more as indicators of the hazard response in fish to the aquatic toxicology [70]. Exposure of Nile tilapia to metals showed reduction in Hb, HTC and RBCs values. This results in harmony with Kaoud et al., [71]. Shah, [72] found decline of Hb values of fish exposed to Pb toxicity and suggested these findings could be related to hemolytic crisis caused by HMs mixture. Also, Naz et al., [73] recorded significant reduction of RBCs, Hb, and hematocrit Hct in different fish species exposed to metals including Cd and Pb. Ullah et al., [74] explained the decrease in RBCs, Hb, and HTC in fish exposed to Cd could result from inhibited hemosynthesis or erythropoiesis, or destruction in hematopoietic tissues. Many researches assumed that exposure to Pb induces disruption of hemoglobin synthesis and may lead to anemia [75, 76, 77, 78]. Also, it may accredited to erythropoiesis, hemosynthesis, and osmoregulatory dysfunction and/or to an elevation the rate of erythrocyte damage in hematopoietic organs because of pollution [79]. Increase in WBCs content in HM-mix group agree with studies recorded increase in WBCs of Nile tilapia exposed to Cd stress [21] and Pb- stress [80]. Several studies recorded the increase in WBCs count in different species exposed to metals' combinations [81,72,82, 83]. As the WBCs are attending for defense, alterations of WBCs count documented in metals exposed- fish to specifies immuno- modulation of poisons [84], and/or organs damage [74]. Gaber et al.[85] assumed this is owing to the immune and protective response, lymphopoiesis stimulation and/or releasing of lymphocytes from lymphoid tissue in presence of metals. QS groups showed a significant increase in these parameters compared with HM- mix group. Fish reared in QS_{150} group recorded the lower WBCs count as the control values. Levavi-sivan et al.[65] showed strong

hemolytic activities of QS on the human blood. Francis et al.[86] informed that there is positive, and others negative QS influences on blood indices. The positive effect might be because of increased oxygen uptake by the fish with a concomitant increase in Hb, RBCs and HTC levels, after the fish were exposed to QS.

Tilapia liver is approximately the best sensitive organ where it appears changes in biochemical features subsequently to the contact with different toxins [87]. It is the target for various water pollutants, including HMs [22]. In the present study, activity of measured enzymes revealed abnormal activities when fish exposed to metals for 40 days (in comparing with the control group). This is concord with many previous studies reported elevation in the fish liver function enzymes (AST, ALT and ALP) exposed to toxicity of metals [88, 21, 89]. Stress might elevate the permeability of animal cell membranes, producing higher enzymes' activities [21] by outflow into the blood stream [90, 91]. Fish of QS_{150} and QS_{300} have significantly enhanced the activities of these enzymes. Fleck et al.[29] informed that QS noticeably decreased the ALT activity (57%), AST (66%) and ALP (76%). Fish received 500 mg QS displayed enhancement of values of total protein and globulin. Elkaradawy et al.[26] and Elkaradawy et al.[67] revealed that QS significantly increased the total protein values in received Nile tilapia. Proteins have important functions as a nitrogenous metabolism and energy source (in stress) and construct the cell structural elements [74]. This might be accompanying the suppressed oxidative metabolism in the fish and hypoxia [36,74]. Lack of produced energy and hypoxia cause respiratory distress because of the buildup of lactic acid. Accordingly, under stress, respiratory pathway would have changed from aerobic to anaerobic. Fish obligates to use proteins as an alternative energy source in case of limited carbohydrates [74]. The improvement in total protein is owing to the augmented secretion of trypsin in the fish received QS. Urea, creatinine and bilirubin are nitrogenous wastes [92]. Increase the levels of urea have been used as nephrotoxicity indicators [22]. Bilirubin is an excretory catabolic product of hemoglobin and its higher levels may cause hypobilirubinea [93]. Fish exposed to pollutants verified higher increase of urea and bilirubin values [22] as a result of generation of various pathological activities in the fish tissues in presence of metals [48]. Different QS supplementations showed decrease levels of these products (specially QS_{150}) in comparing with HM-mix group. Abdel-Reheim et al.[35] documented rates fed with QS supplementation diet have had a lower bilirubin value (54%). Fleck et al.[29] assumed the reason is the hepatoprotective effect of QS which probably owing to the enhancement of oxidative status and preserving hepatocyte integrity and functioning. The elevation of the lipid energy investments in response to contamination [85]. The excess energy funds (as glucose, triglycerides, and cholesterol) are consumed to mediate the effects of stress. As homeostasis of lipids is regarded one of the main liver functions [85]. The principal function of triglycerides is storing and providing the cellular energy and they are used to estimate growth status and lipid metabolism [94]. Elkaradawy et al.[67] showed significant decrease of triglycerides analyzed in Nile tilapia received QS in comparing with the control group. Deng et al.[34] showed similar effect on Hybrid groupers fed with steroid saponins supplementations. Abdel-Reheim et al.[35] showed decrease in triglycerides of stressed rates to 70% when received QS additives. Abdel-Reheim et al.[35] and Elkaradawy et al.[67] explained the decrease in triglycerides owing to the reduction in intestinal cholesterol absorption and the saponins' protective activity. In addition, growth in the β-hydroxy β- methylglutaryl- CoA (HMG- CoA) reductase action and low- density lipoprotein receptor quantities in the liver. Unlike, Sørensen et al.[95] found that there were no significant changes in plasma triglycerides in Atlantic salmon fed with soya-saponins. HDL values were seriously affected in the presence of metals. This could be accompanying the energy requires in addition the serum carriage of fatty acids through HDLs [96]. Elbialy et al.[36] found that *Yucca schidigera* extract added to stressed-fish diet improved the HDL levels. Deng et al.[34] explained the shift in HDL values with steroid saponins by way of their ability for improvement the triglycerides breakdown to fatty acids through activating the PPAR signaling path. This stimulates the LDL and HDL receptors families within the cholesterol metabolic path to mediate cholesterol transport. The findings of the present research could indicate that supplementation with 300 and 150 mg QS/kg fish feed may play a significant role in protection of liver. The improved protection of stressed fish liver could have a health, welfare and profitable impact.

Oxidative stress may induces damaging mitochondrial respiration, increasing reactive oxygen species (ROS) generation, promoting lipid peroxidation (LPO is considered one of the major activities stimulated by oxidative stress), and reducing intracellular antioxidants within target cells [97]. The antioxidant enzyme system is a potential biochemical biomarker in environmental biomonitoring remarkably in response to metal pollution [74]. According to our findings, Cd and Pb

decreased the analyzed SOD and GSH. Antioxidants are potential objectives for Pb. Most antioxidants have thiol groups on their active sites and Pb has a high affinity for these groups. So, Pb can fix producing deactivation of the enzyme [98]. The depletion of SOD and GSH and increase of MDA activities is well-matched with previous studies [99, 100, 101]. Our findings also concord with Rajeshkumar et al.[102] who experimented Cd and Pb impacts on antioxidants of common carp for 30 days. Higher MDA levels could be related to the metal-induced ROS generation which would have stimulate LPO production creating loss of cell membrane fluidity in oxidative stress [22]. Wang et al.[88] explained the significant elevation of MDA levels ($p < 0.05$) after 24 h exposure to 10 µM Cd as Cd induced LPO in the hepatocytes. MDA offers a relative measure of prospective oxidative damage caused by toxins [103]. SOD and CAT are crucial enzymes that counteract the harmful impacts of oxygen metabolism [88] and safeguard the organism's body [104, 105]. Operating in a highly coordinated system, these enzymes play a pivotal role in converting reactive oxygen species into non-toxic oxygen within the liver [102, 106]. SOD transforms superoxide anions (O_2–) to less toxic components, mainly H_2O_2 and O_2 [107]. Next, CAT catalyzes H_2O_2 to water and molecular oxygen [108, 109]. GSH performances as a free radical scavenger, supports the stimulation of other antioxidants and acts as an electron donor in the peroxides reduction mechanism managed by GPx [110]. It is a non-enzymatic antioxidant, works as a reducing agent with xenobiotic conjugation [82], shield against metal cations [111]. Environmental toxicity can affect these enzymes activities causing oxidative stress [21]. Their combined behavior facilitates elimination of the free radicals, and in the standard physiological conditions, a delicate equilibrium is between these components. but, in cases of excessive free radicals' generation, this balance is interrupted, causing oxidative insult [88]. Effects of different concentrations of QS on antioxidants enzymes activities were found as following: QS_{150}> QS_{300} > QS_{500}. QS extract has been reported to contain a combination of antioxidants [112]. Elbialy et al., [36] informed that yucca extract has saponins which performance as natural antioxidants. This study is in concord with Elkaradawy et al.[26] who found that QS supplementations to Nile tilapia fish diet enhanced SOD and CAT levels. Deng et al.[34] recorded those groupers fed diets comprising 0.1% steroidal saponins showed significant increase in SOD, CAT and GSH levels and decrease in serum MDA levels. Fleck et al.[29] supposed that the ability of QS to scavenge excessive radicals and consequently defeat oxidative stress is the motive for depletion of liver MDA production. The depletion of hepatic MDA was owing to releasing of ROS (in hypoxia condition), which improved LPO [113].

Cholinesterase is as one of the select neuro-biochemical indices for the estimation of environmental stress [82] by regulating nerve impulses transmission via cholinergic synapses [114, 115]. It is used previously as a biomarker of neurotoxic injures in aquatic pollution studies [116]. Metal ions are studied as neurotoxic compounds with potential inhibition of ChE enzymes activity [117, 92, 115]. Water pollution induces possible toxicity reducing ChE enzyme described as "anticholinesterase agents", which is a key factor on the neurotransmitter acetylcholine [82]. Chandrasekera et al.[118] recorded decrease of ChE percent in brain and muscle by 24-32% and 33-35% respectively of *Oreochromis niloticus* exposed to Cd^{2+} (1 mg/L). Sabullah et al.[119] found that Cd and Pb reduced ChE activity in *Puntius Javanicus* in vitro to 49.14%, 88.68% respectively. Richetti et al.[120] examined significant decrease in acetylcholinesterase of zebrafish has been exposed to lead acetate. Ullah et al.[74] found that *Tor putitora* collected from metals-polluted river sites has a significant reduction in the muscles and liver acetylcholinesterase activity unlike to fish collected from the reference sites. In the present study, Cd and Pb mixture decreased the activity of ChE to about 37.5%. The inhibition might be associated with metals bioaccumulation [74]. Cogun et al.[79] informed that fish serum ChE activity could be a more liver dysfunction specific indicator. He observed a drop in ChE synthesis beside hepatocyte dysfunction because of mercury exposure afterward synthesis restoration with hepatocyte recovery. QS succeeded in restoration of ChE activity and increased its levels to 90% - 91.56% in presence of metals.

Cytochrome *CYP*, is supported monooxygenase induction that approximately used as a biomarker of exposure to pollution as polyaromatic hydrocarbons, polychlorinated biphenyls [121,116,122] and pesticides [123]. It is a hepatic protein sensitive of natural detoxification processes [124]. *CYP P450* has been employed as a susceptible biomarker for monitoring fish exposure to environmental poisons [116]. It is a chief by-product in oxidative stress cases when the free radicals generation during the organism exposed to pollutants [123]. *Cyp19* is found to up-regular activate in cases of tissues injuries [125]. *GPx* is one of the antioxidative component which has a significant role in reduction of the oxidative stress by degenerating the free radicals, producing high antioxidative and immune responses [126, 127]. Fish of QS_{300} and QS_{150} groups

revealed restoration of *CYP* and *GPx* balance of cell proliferation and apoptosis through up-regulation of *GPx* together with down-regulation of *CYP19* to impede tumor initiation and progression.

Nile tilapia gills are the most important portion exposed to metals which in close contact with the water and only a few micrometers separate the blood from the external medium [128]. In the present study, exposure to metals showed necrosis of gill lamellae and this observation agree with previously documents [129,71,73]. In presence of QS, less damage observed on gills. Elkaradawy et al.[26] recorded decrease of gills necrosis, degeneration, and inflammation of Nile tilapia fingerlings. This may because fish of QS has improvement of hematological indices and feeding efficiency. As liver is the detoxification site in the fish body, exposure to Cd and Pb showed hypertrophy and hyperplasia of bill duct cells, eosinophilic debris. Our results agree with many previous studies [130, 102, 12]. Assimilation of QS on fish exposed to metals showed few congested sinusoids and vasculature and a few inflammatory cell infiltrations of hepatocytes. Enhancing of liver enzymes as MDA, SOD and GSH alleviate the liver damage. Deng et al.[34] observed the ability of steroid saponin diets in enhancing the hybrid groupers liver damage. Our result also in concord with Abdel-Reheim et al.[35] and Abdel-Reheim et al.[131] who recorded the same results in rats. Fish spleen recorded high sensitivity in presence of metals [132, 133, 134]. Higher absorbance of QS in intestine may be the cause of the relatively normal appearance of splenic tissue. Previous studies recorded gliosis and meningitis of fish brain exposed to metals [129]. This may because of Pb is known to bioaccumulate in the brain [135]. No available data about the effect of QS on the histology of the fish brain. Enhancement of the cholinesterase activities mirrored in the histopathological healthy brain of the fish. In the present study, fish received QS showed normal architecture of molecular layer with low degeneration.

Conclusion

Our study work has shown that QS has diminish effect against Cd and Pb negative impacts on fish. QS concentrations 150, 300 and 500 mg/kg fish feed enhanced growth performance, haematological and biochemical indices of fish reared in aquarium containing heavy metals. 150 mg QS/kg fish feed hindered the oxidative stress. It also achieved, especially 500 mg QS/kg, cell balance of proliferation and apoptosis. Therefore, we can recommend QS as a promising feed additive to improve therapeutic substitutes caused by metals stress.

Acknowledgements

The authors would like to thank the Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt, for providing facilities to carry out this experiment.

Data Availability Statement

All relevant data are available from the authors upon request.

Author contributions

All authors contributed equally to this work (conception, acquisition, samples analysis, statistical analysis, data interpretation, manuscript drafting, and manuscript revision).

Funding Information

This study was not financially supported by any funding organization.

Conflict of interest

The authors declare that they have no conflict of interest.

TABLE 1. Primers used for qRT-PCR analysis.

Gene	Primer	Reference
CYP19	F: CGTCATGTTGCTTCTCATCG R: TACCGCAGGCTCTCGTTAAT	Bois et al.[62]
GPx	F: TCGGACATCAGGAGAACTGC R: GCACTGCTCAAAGTTCCAGG	Agus et al.[63]
*B-actin**	F: CACACAGTGCCCATCTACGA R: CCACGCTCTGTCAGGATCTTCA	Agus et al.[63]

CYP19: Cytochrome, *GPx*: glutathione peroxidase, *housekeeping gene.

TABLE 2. Growth performance and feed utilization efficiency of Nile tilapia challenged with heavy metals mixture and treatment with *Quillaja Saponaria.*

Parameters	C-G	HM-mix	QS_{500}	QS_{300}	QS_{150}	p-value
IBW (g)	60.05±1.775	62.25±2.865	63.4±2.272	61.03±2.299	62.18±2.868	0.8947
FBW (g)	107.7±3.07 [a]	89.13±3.935 [b]	109.9±2.161 [a]	104.8±3.202 [a]	105.4±2.56 [a]	0.0028
WG (g)	45.51±1.038 [a]	28.29±0.7188 [b]	49.28±0.575 [a]	46.85±0.8725 [a]	43.5±1.328 [a]	0.0001
WG%	75.79±1.728 [a]	45.6±1.162 [b]	77.72±0.9069 [a]	76.77±1.434 [a]	69.96±2.131 [a]	0.0001
FCR	1.584±0.036 [a]	2.107±0.053 [b]	1.544±0.018 [a]	1.564±0.029 [a]	1.717±0.052 [a]	0.0008
SGR %/day	1.46±0.024 [a]	0.9048±0.054 [b]	1.376±0.049 [a]	1.351±0.052 [a]	1.32±0.036 [a]	0.002
Survival rate	100±0.00	95.84±4.165	100±0.00	95.84±4.165	100±0.00	0.7392

IBW: initial body weight, FBW: final body weight, WG %: weight gain percent, WG: weight gain, FCR: Feed conversion ratio, SGR: Specific growth rate. Note: Means within the same row lack common superscripts are significantly different at $p < 0.05$, data expressed as mean ± SEM.

TABLE 3. Hematological parameters of Nile tilapia challenged with heavy metals mixture and treatment with *Quillaja Saponaria*

Parameters	C-G	HM-mix	QS_{500}	QS_{300}	QS_{150}	p-value
Hb (g/100l)	11.50±0.394 [a]	8.18±1.420 [b]	10.57±0.731 [a]	8.27±0.4041 [b]	10.50±0.625 [ab]	0.0216
HCT (%)	28.57±1.733 [a]	18.73±1.977 [b]	26.03±1.658 [ab]	18.53±0.504 [b]	25.57±2.404 [ab]	0.007
RBCs (x 10^6/mm^3)	1.77±0.088 [a]	1.167±0.066 [b]	1.7±0.115 [a]	1.2±0.057 [b]	1.7±0.057 [a]	0.0005
MCH (pg)	60.04±3.055	59.33±2.028	63±1.000	65.67±0.333	61±1.528	0.1329
MCHC (%)	40.67±1.202	40.67±1.041	41.00±0.577	43.33±0.882	44.33±0.882	0.0458
WBCs (x10^3/ µl)	63.13±9.255 [a]	94.57±2.123 [b]	78.03±5.951 [ab]	70.87±2.577 [ab]	68.50±5.020 [a]	0.0142
Lymphocytes (×10^3/µl)	63.00±6.506	77.67±2.848	73.67±4.177	75.67±4.702	70.33±7.881	0.4143
Neutrophils (×10^3/µl)	7.67±2.848	8.67±1.202	10.67±0.333	9.67±4.702	9.33±2.848	0.9344
Monocytes (×10^3/µl)	14.00±1.528	10.33±1.856	12.67±1.202	8.33±1.333	14.33±2.906	0.1925
Eosinophils (×10^3/µl)	4.00±1.155	3.33±1.333	4.67±0.882	2.67±0.333	4.33±1.453	0.7186

WBCs: white blood cell count, RBCs: Red blood cells count, Hb: hemoglobin content, HCT: hematocrit, MCH: mean corpuscular haemoglobin and MCHC: mean corpuscular haemoglobin concentration. Note: Means within the same row lack common superscripts are significantly different at $p < 0.05$, data expressed as mean ± SEM.

TABLE 4. Serum biochemical- profile of Nile tilapia challenged with heavy metals mixture and treatment with *Quillaja Saponaria*

Parameters	C-G	HM-mix	QS_{500}	QS_{300}	QS_{150}	p-value
ALT (U/L)	32.33±4.807^{a}	54.33±1.764^{b}	82.33±6.333^{c}	20.67±3.18^{a}	37.33±3.93^{ab}	0.0001
AST (U/L)	59±5.292^{a}	260±29.51^{b}	184±14.47^{c}	22.67±3.712^{a}	43±3.786^{a}	0.0001
Albumin (g/dl)	1.267±0.088	1.02±0.057	1.133±0.088	1.133±0.033	1.0±0.057	0.1782
Total protein (g/dl)	3.367±0.145^{a}	2.533±0.202^{b}	3.367±0.145^{a}	3.333±0.12^{a}	2.233±0.218^{b}	0.0015
Globulin (g/dl)	2.10±0.152^{a}	1.433±0.233^{b}	2.233±0.133^{ab}	2.2±0.115^{ab}	1.233±0.218^{c}	0.0057
Urea (mg/dl)	21±1.155^{acd}	26±0.574^{b}	21.33±1.202^{ac}	17.33±0.333^{d}	16.33±0.667^{d}	0.0001
Creatinine (mg/dl)	0.2333±0.218^{a}	0.333±0.088^{a}	0.2±0.03^{a}	0.3±0.054^{a}	0.133±0.033^{b}	0.0208
ALP (mg/dl)	118.3±6.566^{ac}	157.3±10.27^{b}	134±9.609^{ab}	100±7.155^{c}	96.67±3.383^{c}	0.0007
Triglycerides (mg/dl)	241.7±11.14^{ac}	229±22.3^{ac}	295.3±3.844^{a}	191±3.055^{bc}	156.3±15.92^{b}	0.0003
Total lipid (mg/dl)	513.7±14.34^{a}	589.7±18.35^{b}	580.3±20.63^{b}	307±1.732^{c}	260.7±3.48^{c}	0.0001
HDL (mg/dl)	30.67±0.881^{a}	23±1.155^{b}	20.33±0.889^{b}	32±1.155^{a}	34.33±0.881^{a}	0.0001
Glucose (mg/dl)	31.67±1.453^{a}	35±1.155^{b}	30.33±0.881^{ab}	28.33±0.882^{a}	29.33±1.856^{ab}	0.0353
Cholesterols (mg/dl)	169±21.03	200.3 ±6.119	196±8.386	196.7±7.265	176.7±7.881	0.2833
Bilirubin (mg/dl)	0.016±0.006^{a}	0.06±0.005^{b}	0.05±0.005^{b}	0.04±0.005^{ab}	0.036±0.008^{ab}	0.0104

AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline triphosphatase and HDL: High density lipoprotein Note: Means within the same row lack common superscripts are significantly different at $p < 0.05$, data expressed as mean ± SEM.

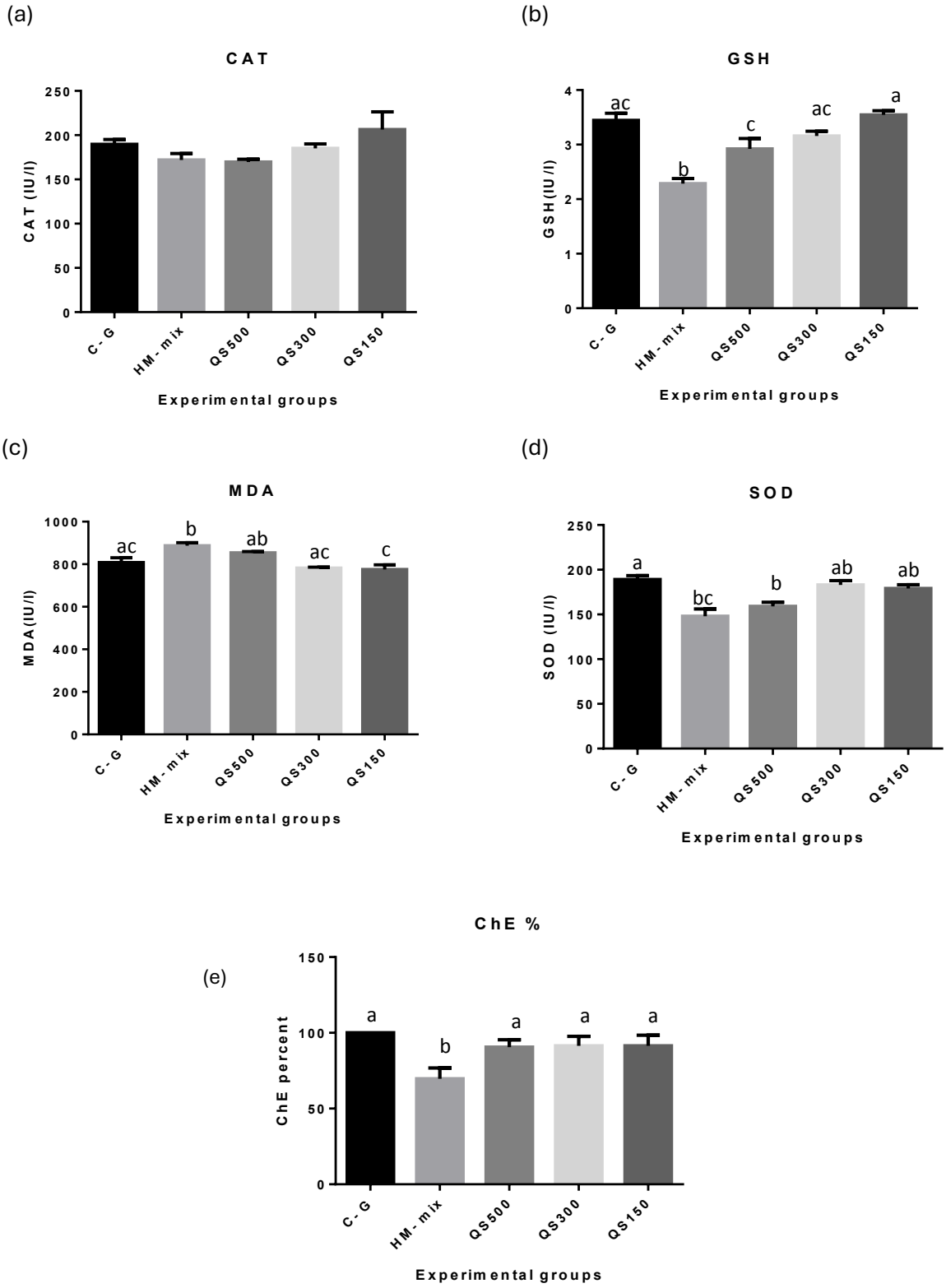

Fig. 1 Oxidative stress assay parameters: (a): CAT (catalase), (b): GSH (glutathione), (c): MDA (malondialdehyde), (d) SOD (superoxide dismutase), (e) ChE % (cholinesterase percent). values are expressed on columns (mean ± SEM) and different superscripts are significantly different ($p < 0.05$) using one- way ANOVA analysis.

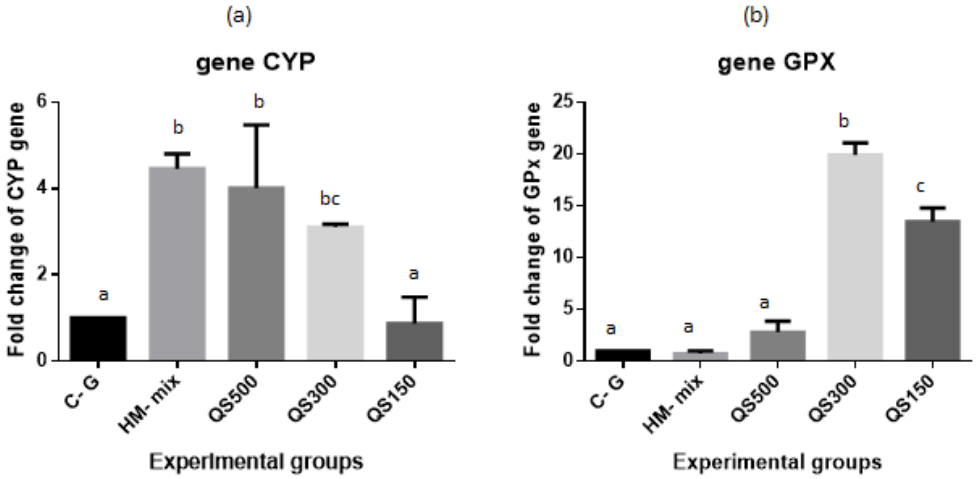

Fig. 2. Relative mRNA expressions of (a) *CYP* (cytochrome) gene and (b) *GPx* (glutathione peroxidase) gene in Nile tilapia liver. Transcriptomic levels are expressed on columns (mean ± SEM) and different superscripts are significantly different ($p < 0.05$) using one- way ANOVA analysis.

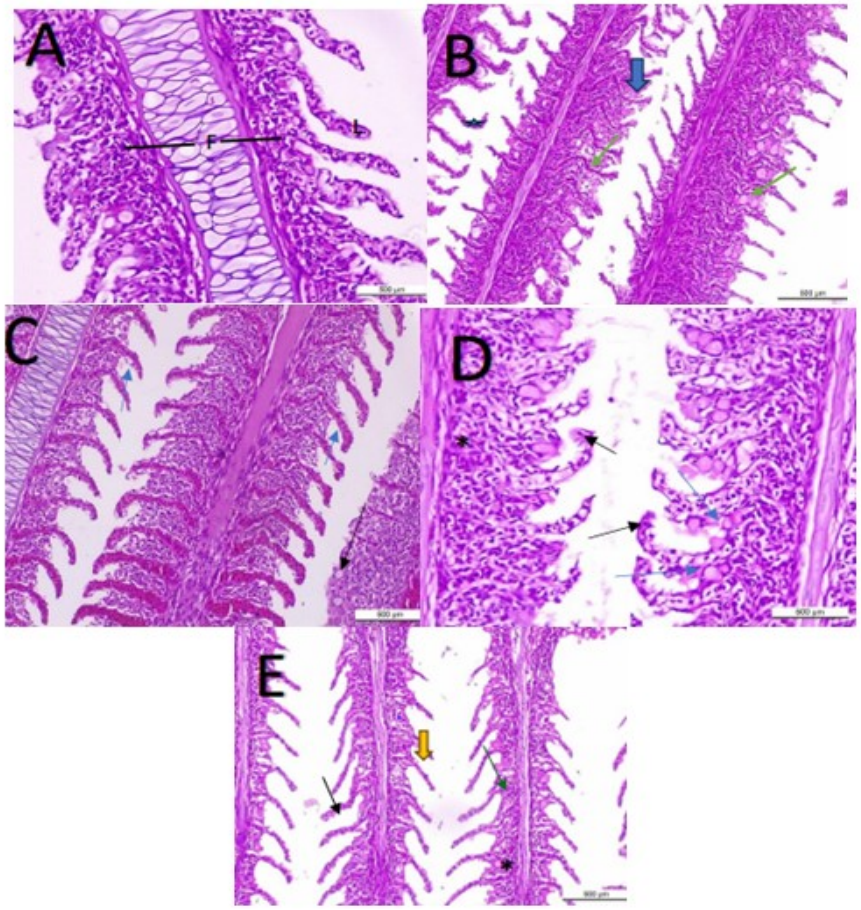

Fig. 3. Photomicrograph of a transverse section of H & E- stained Nile tilapia gill filaments (X 400). (A) Control fish, showing normal appearance of gill filaments (F) and lamellae (L). (B) gills exposed to metals showing hyperplasia leading to shortening secondary lamella with congested blood vessels (blue arrow), hypertrophy of chloride cells and mucus cells (green arrow), curling of secondary lamella (*). (C) Gills treated with150 mg *Quillaja Saponiria*/ kg fish feed look healthier and there is no adhesion between the filaments (blue arrow) and a few hypertrophies of chloride cells. (D) and (E) Gills treated with 300, and 500 mg *Quillaja Saponiria*/ kg fish feed showed hypertrophies of chloride cells (green arrow), curling of secondary lamella (black arrow), and decreased adhesion of secondary gill lamella (blue arrow) with eosinophilic granular cells (yellow arrow).

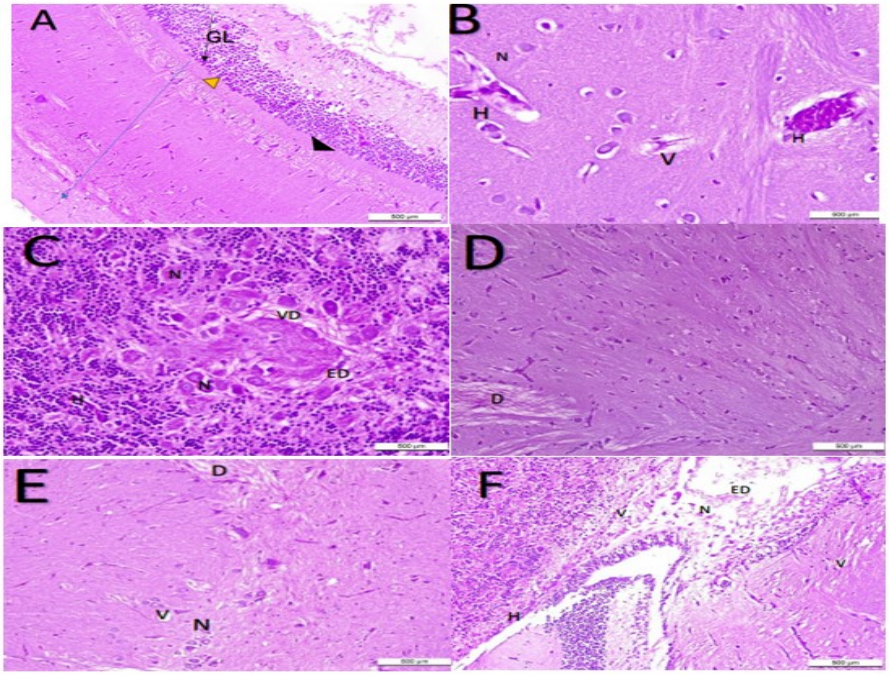

Fig. 4. Photomicrograph of brain section of the Nile tilapia (Stain: H&E; Magnification: ×400). A; control group granular layer (GL), molecular layer (ML), ganglion layer (short arrow), and Purkinje cells (orange arrowheads). (B& C): Fish exposed a mixture of metals showing degenerations (D), vacuolar degeneration (VD), edema (ED), necrosis (N), and hemorrhage (H). fish exposed to 150 µg saponin showed normal architecture of molecular layer with low degeneration. Figure (E) Fish exposed to 300 µg saponin showed vacuolar degeneration (VD), edema (ED), necrosis (N), and hemorrhage (H). Fish treated with saponin 500µg showed a separation of the granular layer from the molecular layer, focal necrosis, vacuolation in the granular and molecular layers, degeneration of the granular layer, and the emergence of edema in multiple regions of the nervous tissue. There was also bleeding between the components of this tissue.

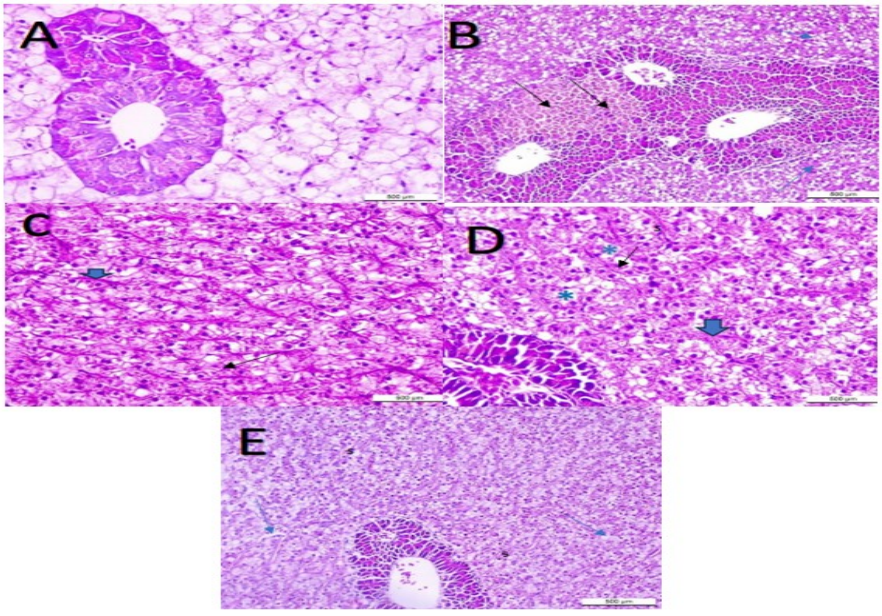

Fig.5. Microscopic examination of H&E-stained Nile tilapia liver sections with representative microphotographs. A: control group; showing normal liver have well-arranged cords of normal hepatocytes, B: liver of fish exposed to metals; showing hypertrophy and hyperplasia of bill duct cells (blue arrow), eosinophilic debris (black arrow). C and D: liver of fish exposed to 500 and 300 mg *Quillaja Saponiria*, respectively; showing Showing a few inflammatory cell infiltrations (blue arrow), vaculation (*) and few necrosis (black arrow). E: liver of fish exposed to 150 mg *Quillaja Saponiria* showing well-organized architecture of hepatic lobules with few congested sinusoids (s) and vasculature, a few inflammatory cell infiltration, and mild sporadic vacuolar degenerative changes (blue arrow) of hepatocytes

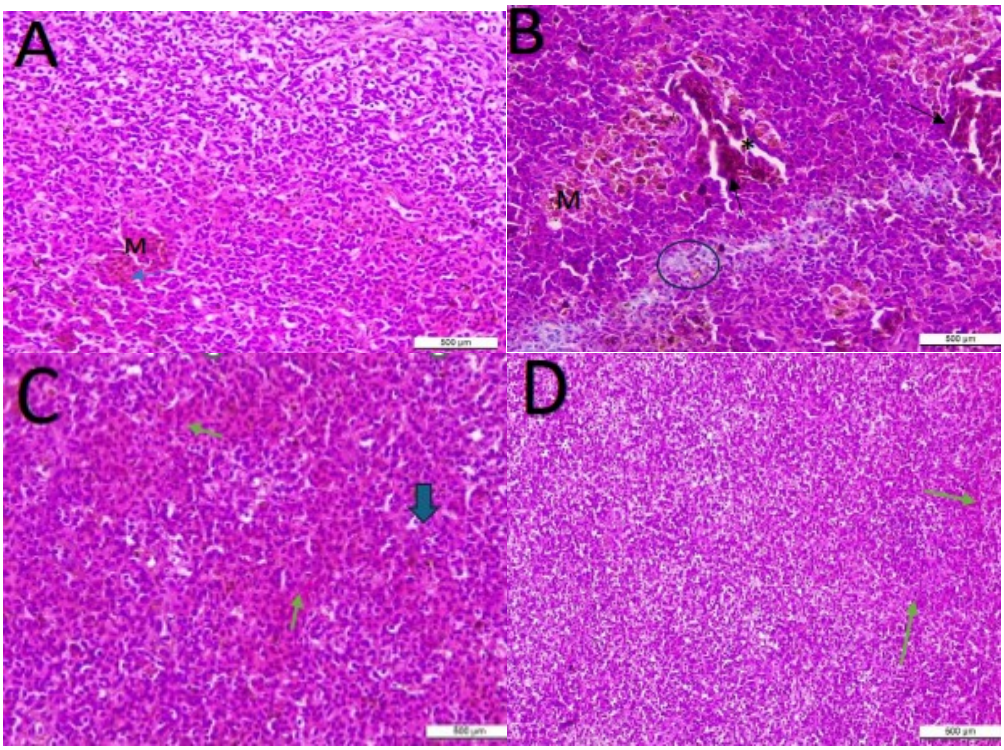

Fig.6. photomicrographs of splenic tissues (H & E stain, scale bar = 500 µm) of Nile tilapia. A; The control group; showed normal splenic structures, melanomacrophage aggregation. B; fish exposed mixture of metals, splenic tissue showed increasing melanomacrophage aggregation (M), necrosis (*) eosinophilic infiltrate (black arrow) and white pulp (circle). C and D; Fish treated with Quillaja Saponiria The splenic tissue has a relatively normal appearance with increased lymphocytic infiltration (green arrow) and hemorrhage (short arrow). There are no significant differences between different concentrations.

References

1. Singh, A., Sharma, A., Verma, R.K., Chopade ,R.L., Pandit, P.P., Nagar, V., Aseri, V., Choudhary, S.K., Awasthi, G., Awasthi, K.K. and Sankhla, M.S. Heavy Metal Contamination of Water and Their Toxic Effect on Living Organisms in Dorta, D.J. and Oliveira, D.P. de (eds.). *The Toxicity of Environmental Pollutants*. Ch. 2. IntechOpen, Rijeka (2022)
2. Swain, C.K. Environmental pollution indices : a review on concentration of heavy metals in air , water , and soil near industrialization and urbanisation. *Discov. Environ.*, **2**(1), 1-14 (2024)
3. Ju, Y.R., Chen, C.F.C.W., Chen, C.F.C.W., Chuang, X.Y. and Dong, C. Di Assessment of heavy metals in aquaculture fishes collected from southwest coast of Taiwan and human consumption risk. *Int. Biodeterior. Biodegrad.*, **124**, 314–325 (2017)
4. Mondal, M., Kumar, J., Fai, Y., Sarkar, B., Sarkar, D., Rai, M., Kumar, S. and Hooda, P.S. Chemosphere A wastewater bacterium Bacillus sp . KUJM2 acts as an agent for remediation of potentially toxic elements and promoter of plant (Lens *culinaris*) growth. *Chemosphere*, **232**, 439–452 (2019)
5. Ahmed, K.M., Baki, M.A. and Kundu, G.K., Islam , S.M., Islam, M.M., Hossain, M.M. Human health risks from heavy metals in fish of Buriganga river, *Bangladesh Springerplus*, **5**, 0–12 (2016)
6. Saha, N., Mollah, M.Z.I.I., Alam, M.F., Safiur Rahman, M. and Sa, M. Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. *Food Control*, **70**, 110–118 (2016)
7. Shaaban, N.A., Tawfik, S., El-Tarras, W. and Ali, T.E.S. Are The Water Quality of the Agricultural Drainage and Nile River Suitable for Tilapia Culture? A Case Study from Kafr El-Shaikh's Fish Farms, Egypt. *Turkish J. Fish. Aquat. Sci.*, **22**(11), 21395 (2022)
8. Achary, M.S., Satpathy, K.K., Panigrahi, S., Mohanty, A.K., Padhi, R.K., Biswas, S., Prabhu, R.K., Vijayalakshmi, S. and Panigrahy, R.C. Concentration of heavy metals in the food chain components of the nearshore coastal waters of Kalpakkam, southeast coast of India. *Food Control*, **72**, 232–243 (2017).
9. Abdel-Tawwab, M., El-Sayed, G.O. and Shady, S.H. Growth, biochemical variables, and zinc bioaccumulation in Nile tilapia, *Oreochromis niloticus* (L.) as affected by water-born zinc toxicity and exposure period. *Int. Aquat. Res.*, **8**, 197–206 (2016)
10. Oyetibo, G.O., Miyauchi, K., Huang, Y., Chien, M.F., Ilori, M.O., Amund, O.O. and Endo, G. Biotechnological remedies for the estuarine environment polluted with heavy metals and persistent organic pollutants. *Int. Biodeterior. Biodegrad.*, **119**, 614–625 (2017)

11. Rahman, A. and Sdeek, F. Monitoring and minimizing levels of some heavy metals in groundwa- ter and water purification stations samples in El-Gharbiya Egypt. *Sci. J. Pestic.,* **3**, 28–37 (2017)

12. Shaaban, N.A., Tawfik, S., El-Tarras, W. and El-Sayed, A., T. Potential health risk assessment of some bioaccumulated metals in Nile tilapia (Oreochromis niloticus) cultured in Kafr El-Shaikh farms, Egypt. *Environ. Res.*, **200**, 111358 (2021)

13. Chatta, A.M., Khan, M.N., Mirza, Z.S. and Ali, A. Heavy metal (Cadmium, lead, and chromium) contamination in farmed fish: A potential risk for consumers' health. *Turkish J. Zool.*, **40**, 248–256 (2016)

14. Al-Kenawy, D.A. and Aly, N.A.F. Levels of Some Heavy Metals in Muscles and Liver of Freshwater Farmed Fish at Abbassa. *Abbassa Int. J. Aqua.*, **8**, 20–35 (2015)

15. Afshan, S., Ali, S., Ameen, U.S., Farid, M., Bharwana, S.A., Hannan, F. and Ahmad, R. Effect of different heavy metal pollution on fish. *Res. J. Chem. Environ. Sci.*, **2**, 74–79 (2014)

16. Abdel-mohsien, H.S. and Mahmoud, M.A.M. Accumulation of Some Heavy Metals in Oreochromis niloticus from the Nile in Egypt : Potential Hazards to Fish and Consumers. *J. Environ. Prot. (Irvine,. Calif).*, **6,** 1003–1013 (2015)

17. Dadar, M., Adel, M., Ferrante, M., Nasrollahzadeh Saravi, H., Copat, C. and Oliveri Conti, G. Potential risk assessment of trace metals accumulation in food, water and edible tissue of rainbow trout (*Oncorhynchus mykiss*) farmed in Haraz River, northern Iran. *Toxin Rev.*, **35**, 141–146 (2016)

18. Álvarez-González, C.A., Martínez-Sánchez, L., Peña-Marín, E.S., Guerrero-Zárate, R., Jesús-Ramírez, F., Morales-García, V., Uribe-López, M. and Núñez-Nogueira, G. Effects on the Growth and Digestive Enzyme Activity in Nile Tilapia Fry (*Oreochromis niloticus*) by Lead Exposure. *Water, Air, Soil Pollut.*, **231**, 1-15 (2020)

19. Yirgu, Z. Accumulation of Certain Heavy Metals in Nile Tilapia (*Oreochromis niloticus*) Fish Species Relative to Heavy Metal Concentrations in the Water of Lake Hawassa, (Thesis) Addis Ababa University, Ethiopia (2011)

20. Liang, P., Wu, S.C., Zhang, J., Cao, Y., Yu, S. and Wong, M.H. The effects of mariculture on heavy metal distribution in sediments and cultured fish around the Pearl River Delta region, south China. *Chemosphere*, **148**, 171–177 (2016)

21. Qiang, J., Tao, Y.F., He, J., Xu, P., Bao, J.W. and Sun, Y.L. miR-122 promotes hepatic antioxidant defense of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) exposed to cadmium by directly targeting a metallothionein gene. *Aquat. Toxicol.*, **182**, 39–48 (2017)

22. Abdel Rahman, A.N., ElHady, M., Hassanin, M.E. and Mohamed, A.A.-R. Alleviative effects of dietary Indian lotus leaves on heavy metals-induced hepato-renal toxicity, oxidative stress, and histopathological alterations in Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, **509**, 198–208 (2019)

23. El-Kassas, S., Abdo, S.E., Abosheashaa, W., Mohamed, R., Moustafa, E.M., Helal, M.A. and El-Naggar, K. Growth performance, serum lipid profile, intestinal morphometry, and growth and lipid indicator gene expression analysis of mono-sex Nile tilapia fed Moringa oleifera leaf powder. *Aquac. Reports*, **18**, 1-9 (2020)

24. Jumah, Y.U., Tumbokon, B.L.M. and Jr, A.E.S. Dietary Quillaja saponin improves growth and resistance against acute hyposalinity shock in the black tiger shrimp *Penaeus monodon* post larvae. *Isr. J. Aquac.*, **72**, 1–13 (2020)

25. Francis, G., Levavi-Sivan, B., Avitan, A. and Becker, K. Effects of long term feeding of *Quillaja saponins* on sex ratio, muscle and serum cholesterol and LH levels in Nile tilapia (*Oreochromis niloticus* (L.)) Comp. Biochem. Physiol. Part C. *Toxicol. Pharmacol.*, **133**, 593–603 (2002)

26. Elkaradawy, A., Abdel-Rahim, M.M., Albalawi, A.E., Althobaiti, N.A., Abozeid, A.M. and Mohamed, R.A. Synergistic effects of the soapbark tree, *Quillaja saponaria* and Vitamin E on water quality, growth performance, blood health, gills and intestine histomorphology of Nile tilapia, *Oreochromis niloticus* fingerlings. *Aquac. Reports*, **20**, 1-10 (2021)

27. Serrano, A.E. Effects of Quillaja saponins on growth, feed efficiency, digestive enzyme activities and metabolism of common carp (*Cyprinus carpio* L). *Aquac. Nutr.*, **19**, 468–474 (2013)

28. Tang, J., He, J., Liu, T. and Xin, X. Removal of heavy metals with sequential sludge washing techniques using saponin : optimization conditions , kinetics , removal effectiveness , binding. *RSC Adv.*, **7**, 33385–33401 (2017)

29. Fleck, J.D., Betti, A.H., Da Silva, F.P., Troian, E.A., Olivaro, C., Ferreira, F. and Verza, S.G. Saponins from *Quillaja saponaria* and *Quillaja brasiliensis*: Particular Chemical Characteristics and Biological Activities. *Molecules*, **24**, 171 (2019)

30. Tiemi, C., Kayukawa, M., Aurélio, M., Oliveira, S., Kaspchak, E., Bruna, H., Sanchuki, S., Igarashi-mafra, L. and Mafra, M.R. Quillaja bark saponin effects on *Kluyveromyces lactis* β -galactosidase activity and structure. *Food Chem.*, **303**, 1–7 (2020)

31. Cañon-Jones, H., Cortes, H., Castillo-Ruiz, M., Schlotterbeck, T. and Martín, R.S. *Quillaja saponaria* (Molina) extracts inhibit in vitro *Piscirickettsia salmonis* infections. *Animals*, **10**, 1–12 (2020)

32. Rezaei, D.M. Effect of Different Levels of Saponin on Growth Performance and Food Efficiency in Convict Cichlid (*Amatitlania nigrofasciata*). *J. Fish. Aquat. Sci.*, **15**, 22–26 (2019)

33. Francis, G. Effects of low dietary levels of saponins on two common culture fish – common carp (*Cyprinus carpio* L.) and Nile tilapia (*Oreochromis niloticus*, L), PhD thesis, University of Hohenheim, Stuttgart, Germany, (2001)

34. Deng, H., Zhang, J., Yang, Q., Dong, X., Zhang, S., Liang, W., Tan, B. and Chi, S. Effects of Dietary Steroid Saponins on Growth Performance, Serum and Liver Glucose, Lipid Metabolism and Immune Molecules of Hybrid Groupers (♀*Epinephelus fuscoguttatus* × ♂*Epinephelus lanceolatu*) Fed High-Lipid Diets. *Metabolites*, **13**, 1–14 (2023)

35. Abdel-Reheim, M.A., Messiha, B.A.S. and Abo-Saif, A.A. *Quillaja saponaria* bark saponin protects wistar rats against ferrous sulphate-induced oxidative and inflammatory liver damage. *Pharm. Biol.*, **55**, 1972–1983 (2017)

36. Elbialy, Z.I., Rizk, M., Al-Hawary, I.I., Salah, A.S., Mohammed, R.A., Assar, D.H., Almeer, R. and Dawood, M.A.O. *Yucca schidigera* extract mediated the growth performance, hepato-renal function, antioxidative status and histopathological alterations in Nile tilapia (*Oreochromis niloticus*) exposed to hypoxia stress. *Aquac. Res.*, **52**, 1965–1976 (2021)

37. Mohamed, R.A., Elbialy, Z.I., Abd El Latif, A.S., Shukry, M., Assar, D.H., El Nokrashy, A.M., Elsheshtawy, A., Dawood, M.A.O., Paray, B.A., Doan, H. Van, and El-Far, A.H. Dietary clenbuterol modifies the expression of genes involved in the regulation of lipid metabolism and growth in the liver, skeletal muscle, and adipose tissue of Nile tilapia (*Oreochromis niloticus*). *Aquac. Reports*, **17**, 3-7 (2020)

38. Abumourad, I.M.K., Authman, M.M.N. and Abbas, W.T. Heavy Metal Pollution and Metallothionein Expression: A Survey on Egyptian Tilapia Farms. *J. Appl. Sci. Res.*, **9**, 612–619 (2013)

39. Yousef, A. F.., Abdou, M.S. and Moawad, A.A. Molecular charactarization of antimicrobial resistence in Edwardsiella Tarda isolated from diseased tilapa fish in Egypt. *Egypt. J. Vet. Sci.*, 21-32 (2022)

40. El-Naggar, M.M., Salaah, S., Suloma, A., Khalil, M.T. and Emam, W.W.M. Impact of chitosan and chitosan nanoparticles on reducing heavy metals in the Nile tilapia, *Oreochromis niloticus* Egypt. *J. Aquat. Biol. Fish.*, **26**, 859–875 (2022)

41. El-ayyat, M.S., Nasr, E. and Khater, Z.Z.K. Assessment of heavy metals in water, sediment and fish tissues, from Sharkia province, Egypt Egypt. *J. Aquat. Biol. Fish.*, **15**, 125–143 (2011)

42. Abdel-khalek, A.A. Risk assessment, bioaccumulation of metals and histopathological alterations in Nile tilapia (*Oreochromis niloticus*) facing degraded aquatic conditions. *Bull. Environ. Contam. Toxicol.*, **94**, 77–83 (2015)

43. Ghalwash, H.R., Salah, A.S., El-Nokrashy, A.M., Abozeid, A.M., Zaki, V.H. and Mohamed, R.A. Dietary supplementation with Bacillus species improves growth, intestinal histomorphology, innate immunity, antioxidative status and expression of growth and appetite-regulating genes of Nile tilapia fingerlings. *Aquac. Res.,* **53**, 1378–1394 (2022)

44. Abdel-Tawwab, M., El-Sayed, G.O. and Shady, S.H.H. Capability of some agricultural wastes for removing some heavy metals from polluted water stocked in combination with Nile tilapia, *Oreochromis niloticus* (L.). *Int. Aquat. Res.*, **9**, 153–160 (2017)

45. Yeşilbudak, B., Erdem, C. Cadmium accumulation in gill, liver, kidney and muscle tissues of common carp, *Cyprinus carpio*, and Nile tilapia, *Oreochromis niloticus* Bull. Environ. Contam. *Toxicol.*, **92**, 546–550 (2014)

46. Abozahra, N.A., Albalawi, A.E., Althobaiti, N.A., Mohamed, R.A. and Diab, A.M.. Effects of Pomegranate (*Punica granatum*) Peel Methanolic Extract Dietary Supplementation on *Oreochromis niloticus* Performance, Blood Health, Intestine Morphometry and Immunity. *Egypt. J. Vet. Sci.*, 221–236 (2022)

47. Feldman, B., Dougan, S.T., Schier, A.F. and Talbot, W.S. Nodal-related signals establish mesendodermal fate and trunk neural identity in zebrafish. *Curr. Biol.*, **10**, 531–534 (2000)

48. Abdel-tawwab, M., El-sayed, G.O. and Shady, S.H. Effect of dietary active charcoal supplementation on growth performance, biochemical and antioxidant responses, and resistance of Nile tilapia, *Oreochromis niloticus* (L.) to environmental heavy metals exposure. *Aquaculture*, **479**, 17–24 (2017)

49. Anderson, D.P. and Siwicki, A.K. *Basic hematology and serology for fish health programs*, Fish Health Section, Asian Fisheries Society, (1995)

50. Thrall, M., Baker, D. and Lassen, E. *Veterinary haematology and clinical chemistry*.Lippincott Williams and Wilkins, (2004)

51. Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases Am. *J. Clin. Pathol.*, **28**, 56– 63 (1957)

52. Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, T.J. and Schaffer, R. A candidate reference method for determination of total protein in serum. I. Development and validation. *Clin. Chem.*, **27**, 1642–1650 (1981)

53. Dumas, B. and Biggs, H.G. *Standard Methods of Clinical Chemistry*. Ed., Academic Press, New York., (1972)

54. Zöllner, N. and Kirsch, K. Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion Z. Gesamte. *Exp. Med.,* **135**, 545–561 (1962)

55. Fynn-Aikins, K., Hung, S.S.O., Liu, W. and Li, H. Growth, lipogenesis and liver composition of juvenile white sturgeon fed different levels of D-glucose. *Aquaculture*, **105**, 61–72 (1992)

56. Heinegård, D. and Tiderström, G. Determination of serum creatinine by a direct colorimetric method. *Clin. Chim. Acta.*, **43**, 305–310 (1973)

57. Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the

preparative ultracentrifuge. *Clin. Chem.*, **18**, 499–502 (1972)

58. Abo-Raya, M.H., Alshehri, K.M., Abdelhameed, R.F.A., Elbialy, Z.I., Elhady, S.S. and Mohamed, R.A. Assessment of growth-related parameters and immune-biochemical profile of nile tilapia (*Oreochromis niloticus*) fed dietary ulva fasciata extract. *Aquac. Res.*, **52**, 3233–3246 (2021)

59. Habig, W.H., Pabst, M.J. and Jakoby, W.B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, **249**, 7130–7139 (1974)

60. Brodeur, G.M., Nichols, K.E., Plon, S.E., Schiffman, J.D. and Malkin, D. Pediatric Cancer Predisposition and Surveillance: An Overview, and a Tribute to Alfred G. Knudson Jr. *Clin. Cancer Res.*, **23**, (11), e1–e5. (2017)

61. 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**, 402–408 (2001)

62. Bois, C., Delalande, C., Nurmio, M., Parvinen, M., Zanatta, L., Toppari, J. and Carreau, S. Age- and cell-related gene expression of aromatase and estrogen receptors in the rat testis. *J. Mol. Endocrinol.*, **45**, 147–159 (2010)

63. Agus ,H.H., Sümer, S. and Erkoç, F. Toxicity and molecular effects of di-n-butyl phthalate (DBP) on CYP1A, SOD, and GPx in *Cyprinus carpio* (common carp). *Environ. Monit. Assess.*, **187**, 423 (2015)

64. Suvarna, S. and Layton, C. *The hematoxylins and eosin. Bancroft's theory and practice of histological techniques,. Elsevier Health Sciences*, **1**,173– 186 (2012)

65. Levavi-sivan, B., Hedvat, R., Kanias, T., Francis, G., Becker, K. and Kerem, Z. Exposure of tilapia pituitary cells to saponins : Insight into their mechanism of action. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.,* **140**(1), 79-86 (2005)

66. Stadtlander, T. Saponin fractions from fenugreek (*Trigonella foenum-graecum* L.) as dietary supplements for Nile tilapia (*Oreochromis niloticus* L.) and common carp (Cyprinus carpio L.), Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften, pp:1-131 (2012)

67. Elkaradawy, A., Abdel-Rahim, M.M. and Mohamed, R.A. *Quillaja saponaria* and/or linseed oil improved growth performance, water quality, welfare profile and immune-oxidative status of Nile tilapia, *Oreochromis niloticus* fingerlings. *Aquac. Res.*, **53**, 576–589 (2022)

68. Güroy, B., Mantoğlu, S., Merrifield, D.L. and Guroy, D. Effects of dietary Nutrafito Plus on growth, haemotological parameters and total ammonia-nitrogen excretion of juvenile striped catfish *Pangasianodon hypophthalmus*. *Aquac. Res.*, **47**, 1770–1777 (2016)

69. Wang, B., Thompson, K.D., Wangkahart, E., Yamkasem, J., Bondad-Reantaso, M.G., Tattiyapong, P., Jian, J. and Surachetpong, W. Strategies to enhance tilapia immunity to improve their health in aquaculture. *Rev. Aquac.*, **15**, 41–56 (2023)

70. Firat, Ö. and Kargin, F. Individual and combined effects of heavy metals on serum biochemistry of nile tilapia *Oreochromis niloticus*. *Arch. Environ. Contam. Toxicol.*, **58**, 151–157 (2010)

71. Kaoud, H.A. Bioremediation the toxic effects of mercury-exposure in Nile tilapia (*Oreochromis niloticus*) by using Lemna gibba L. *Toxicol. Lett.*, 205-128 (2011)

72. Shah, N., Khisroon, M. and Shah, S.S.A. Assessment of copper , chromium , and lead toxicity in fish (Ctenopharyngodon idella Valenciennes , 1844) through hematological biomarkers. *Environ. Sci. Pollut. Res.*, **27**, 33259–33269 (2020)

73. Naz, S., Hussain, R., Ullah, Q., Manan, A., Chatha, M., Shaheen, A. and Khan, R.U. Toxic effect of some heavy metals on hematology and histopathology of major carp (*Catla catla*). *Environ. Sci. Pollut. Res.*, **28**, 6533–6539 (2021)

74. Ullah, S., Li, Z., Hassan, S., Ahmad, S., Guo, X., Wanghe, K. and Nabi, G. Heavy metals bioaccumulation and subsequent multiple biomarkers based appraisal of toxicity in the critically endangered Tor putitora. *Ecotoxicol. Environ. Saf.*, **228**, 1-14 (2021)

75. Edwards, P.K. The Correlation of the Concentration of Selected Metals Determined in Water and Fish Samples From a Public Pond. *East Tennessee State University* , MS (Master of Science). Program:Chemistry, Date of Award 12-2010 (2010)

76. Khan, M.S., Goel, A.Z.R. and Musarrat, J. *Biomanagement of Metal-Contaminated Soils.Environmental Pollution.* Springer Dordrecht Heidelberg London New York. pp. 523, (2011)

77. Sardar, K., Shafaqat A., Afzal, S., Hameed, S., Fatima S., Shakoor, M.B., Bharwana, S.A. and Tauqeer, H.M. Heavy Metals Contamination and what are the Impacts on Living Organisms Greener. *J. Environ. Manag. Public Saf.*, **2**, 172–179 (2013)

78. El-Kowrany, S.I., El- Zamarany, E.A., El-Nouby, K.A., El-Mehy, D.A., Abo Ali, E.A., Othman, A.A., Salah, W. and El-Ebiary, A.A. Water pollution in the Middle Nile Delta, Egypt: An environmental study. *J. Adv. Res.*, **7**, 781–794 (2016)

79. Cogun, H.Y., Fırat, Ö., Fırat, Ö., Yüzereroğlu, T.A., Gök, G., Kargin, F. and Kötemen, Y. Protective effect of selenium against mercury-induced toxicity on hematological and biochemical parameters of *Oreochromis niloticus*. *J. Biochem. Mol. Toxicol.*, **26**, 117–122 (2012)

80. Adhim, M.H., Zainuddin, A., Putranto, T.W.C., Irawan, B. and Soegianto, A. Effect of sub-lethal lead exposure at different salinities on osmoregulation and hematological changes in tilapia, *Oreochromis niloticus*. *Arch. Polish Fish.*, **25**, 173–185 (2017)

81. Garcia, G.G., Miguel, E.J.L., Gabriel, M.A.L. and Mingala, C.N. The corollary effect of heavy metal accumulation in freshwater ponds on the

hematological profile of Nile Tilapia (*Oreochromis niloticus*). *Environ. Exp. Biol.*, **14**, 69–73 (2016)

82. Hossain, Z., Hossain, M.S., Ema, N.S. and Omri, A. Heavy metal toxicity in Buriganga river alters the immunology of Nile tilapia (*Oreochromis niloticus* L). *Heliyon*, **7**, e08285 (2021)

83. Pal, S. A comparative study on hematological, bioenergetics and oxidative stress indices of fresh water male spiny eel fish (*Mastacembelus armatus*) collected from the Haora and Gomati Rivers of Tripura, Northeast India in relation to heavy metal contamination. *Comp. Clin. Path*., **31**(1), 265–280 (2022).

84. Ucar, A., Özgeriş, F.B., Yeltekin, A.Ç., Parlak, V., Alak, G., Keleş, M.S. and Atamanalp, M. The effect of N-acetylcysteine supplementation on the oxidative stress levels, apoptosis, DNA damage, and hematopoietic effect in pesticide-exposed fish blood. *J. Biochem. Mol. Toxicol*., **33**, (6),e22311 (2019)

85. Gaber, H.S., El-Kasheif, M.A., Ibrahim, S.A. and Authman, M.M.N. Effect of water pollution in El-Rahawy drainage canal on hematology and organs of freshwater fish *Clarias gariepinus*. *World Appl. Sci. J.*, **21**, 329–341 (2013)

86. Francis, G., Makkar, H.P. and Becker, K. Dietary supplementation with a *Quillaja saponin* mixture improves growth performance and metabolic efficiency in common carp (*Cyprinus carpio* L.). *Aquaculture*, **203**, 311–320 (2002)

87. Authman, M.M.N.N. Environmental and experimental studies of aluminium toxicity on the liver of *Oreochromis niloticus* (Linnaeus, 1757) fish. *Life Sci. J.*, **8**, 764–776 (2011)

88. Wang, J., Zhu, H., Liu, X. and Liu, Z. N-acetylcysteine protects against cadmium-induced oxidative stress in rat hepatocytes. *J. Vet. Sci.*, **15**, 485–493 (2014)

89. Alm-Eldeen, A.A., Donia, T. and Alzahaby, S. Comparative study on the toxic effects of some heavy metals on the Nile Tilapia, *Oreochromis niloticus*, in the Middle Delta, Egypt. *Environ. Sci. Pollut. Res.*, **25**, 14636–14646 (2018)

90. Firat, Ö., Cogun, H.Y., Yüzereroğlu, T.A., Gök, G., Firat, Ö., Kargin, F. and Kötemen, Y. A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*. *Fish Physiol. Biochem*., **37**, 657–666 (2011)

91. Abdel-Tawwab, M. Effect of feed availability on susceptibility of Nile tilapia, *Oreochromis niloticus* (L.) to environmental zinc toxicity: Growth performance, biochemical response, and zinc bioaccumulation. *Aquaculture*, **464**, 309–315 (2016)

92. Elsaeidy; I.M., Assem; H.E., Edrees G.M.F. and Khalifa A.S. Multiple biomarkers response in Nile tilapia, *Oreochromis niloticus* (L.) exposed to sublethal concentrations of hexavalent chromium Egypt. *J. Aquat. Biol. Fish.*, **20**, 37–49 (2016)

93. Masih, S.C. Impact of monocrotophos pesticide on serum biochemical profile in freshwater fish, *Cirrhinus mrigala* (Hamilton, 1822). *Int. J. Biol. Innov.*, **03**, 402–406 (2021)

94. Wafeek, M., Shakr, I. and Aly, S.M. Effect of water hyacinth and chlorella on water polluted by heavy metal and the biochemical and pathophysiological response of exposed fish 8th. *Int. Symp. Tilapia Aquac.*, **513**, 549 (2008)

95. Sørensen, M., Penn, M., El-Mowafi, A., Storebakken, T., Chunfang, C., Øverland, M. and Krogdahl, Å. Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar*, L). *Aquaculture*, **314**, 145–152 (2011)

96. Abdelhamid, A.M., Mehrim, A.I., Abdel Hamid, A.A.M. and El-Katan, M.E. Influence of feeding fish the Dried-Treated sewage on physiological responses and histological structure of the liver. *Asian J. Anim. Vet. Adv*., **10**, 295–310 (2015)

97. Zahran, E. and Risha, E. Modulatory role of dietary Chlorella vulgaris powder against arsenic-induced immunotoxicity and oxidative stress in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.*, **41**, 654–662 (2014)

98. Aykin-Burns, N., Franklin, E.A. and Ercal, N. Effects of N-acetylcysteine on lead-exposed PC-12 cells *Arch. Environ. Contam. Toxicol.*, **49**, 119–123 (2005)

99. Wang, L., Lin, S., Li, Z., Yang, D. and Wang, Z. Protective effects of puerarin on experimental chronic lead nephrotoxicity in immature female rats. *Hum. Exp. Toxicol.*, **32**, 172–185 (2013)

100. Jan, A.T., Azam, M., Siddiqui, K., Ali, A., Choi, I. and Haq, Q.M.R. Heavy metals and human health: Mechanistic insight into toxicity and counter defense system of antioxidants. *Int. J. Mol. Sci.*, **16**, 29592–29630 (2015)

101. Rahmani Talatappeh, N., Ranji, N. and Beigi Harchegani, A. The effect of N-acetyl cysteine on oxidative stress and apoptosis in the liver tissue of rats exposed to cadmium. *Arch. Environ. Occup. Heal.*, **76**, 518–525 (2021)

102. Rajeshkumar, S., Liu, Y., Ma, J., Duan, H.Y. and Li, X. Effects of exposure to multiple heavy metals on biochemical and histopathological alterations in common carp, Cyprinus carpio L. *Fish Shellfish Immunol*., **70**, 461–472 (2017)

103. Abdel-khalek, A.A., Kadry, M.A.M., Badran, S.R. and Marie, M.S. Comparative toxicity of copper oxide bulk and nano particles in Nile Tilapia ; *Oreochromis niloticus* : Biochemical and oxidative stress. *J. Basic Appl. Zool.*, **72**, 43–57 (2015)

104. Morcillo, P., Cordero, H., Meseguer, J., Esteban, M.Á. and Cuesta, A. In vitro immunotoxicological effects of heavy metals on European sea bass (*Dicentrarchus labrax* L.) head-kidney leucocytes. *Fish Shellfish Immunol.*, **47**, 245–254 (2015)

105. Liu, H., Wang, S., Cai, Y., Guo, X., Cao, Z., Zhang, Y., Liu, S., Yuan, W., Zhu, W., Zheng, Y., Xie, Z., Guo, W. and Zhou, Y. Fish & Shell fi sh Immunology Dietary administration of Bacillus subtilis HAINUP40

enhances growth , digestive enzyme activities , innate immune responses and disease resistance of tilapia , *Oreochromis niloticus*. *Fish Shellfish Immunol.*, **60**, 326–333 (2017).

106. Taslima, K., Al-Emran, M., Rahman, M.S., Hasan, J., Ferdous, Z., Rohani, M.F. and Shahjahan, M. Impacts of heavy metals on early development, growth and reproduction of fish – A review. *Toxicol. Reports*, **9**, 858–868 (2022).

107. Bharti, S. and Rasool, F. Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on *Channa punctatus* (Bloch) fish. *Toxicol. Reports*, **8**, 443–455 (2021).

108. Hermenean, A., Damache, G., Albu, P., Ardelean, A., Ardelean, G., Puiu Ardelean, D., Horge, M., Nagy, T., Braun, M., Zsuga, M., Kéki, S., Costache, M. and Dinischiotu, A. Histopatological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania. *Ecotoxicol. Environ. Saf.*, **119**, 198–205 (2015)

109. Bernardes, G., Tarouco, F., Eduardo, C. and Berteaux, R. The utilization of sodium bicarbonate , calcium carbonate or hydroxide in biofloc system : water quality , growth performance and oxidative stress of Nile tilapia (*Oreochromis niloticus*). *Aquaculture,* **468**, 10–17 (2017)

110. Puerto, M., Prieto, A.I., Pichardo, S., Moreno, I., Jos, Á., Moyano, R. and Cameán, A.M. Effects of Dietary N-acetylcysteine on the oxidative stress induced in Tilapia (*Oreochromis niloticus*) exposed to a microcystin-producing cyanobacterial water bloom. *Environ. Toxicol. Chem*., **28**, 1679–1686 (2009)

111. Fırat, Ö., Çogun, H.Y., Aslanyavrusu, S. and Kargın, F. Antioxidant responses and metal accumulation in tissues of Nile tilapia *Oreochromis niloticus* under Zn, Cd and Zn + Cd exposures. *J. Appl. Toxicol.*, **29**, 295–301 (2009)

112. Tippel, J., Gies, K., Harbaum-piayda, B., Steffen-heins, A. and Drusch, S. Composition of *Quillaja saponin* extract affects lipid oxidation in oil-in-water emulsions. *Food Chem.*, **221**,386-394 (2016)

113. Agnello, A.C., Bagard, M., Hullebusch, E.D. Van, Esposito, G. and Huguenot, D. Science of the Total Environment Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation , phytoremediation , bioaugmentation and bioaugmentation-assisted phytoremediation. *Sci. Total Environ.*, **563–564**, 693–703 (2016)

114. Bonansea, R.I., Wunderlin, D.A. and Amé, M.V. Behavioral swimming effects and acetylcholinesterase activity changes in Jenynsia multidentata exposed to chlorpyrifos and cypermethrin individually and in mixtures. *Ecotoxicol. Environ. Saf.*, **129**, 311–319 (2016).

115. Rodrigues, A.P. de C., Carvalheira, R.G., Gomes, V. and Arias, A.R.L., Almosny, N.R.P., Castilhos, Z.C. and Bidone, E.D. Acetylcholinesterase Activity in Fish Exposed to Mercury in Guanabara Bay, RJ, Brazil. *Environ. Pollut. Prot.*, **3**(4), 34001 (2018).

116. Gold-Bouchot, G., Zapata-Perez, O., Rodriguez-Fuentes, G., Ceja-Moreno, V., Rio-Garcia, M. del and Chan-Cocom, E. Biomarkers and pollutants in the Nile Tilapia, *Oreochromis niloticus*, in four lakes from San Miguel, Chiapas, Mexico. *Int. J. Environ. Pollut.*, **26**(1-2), 129-141(2006).

117. Oakes, K., Shan, Z. and Kaliaperumal, R. *Nanotechnology for Water Treatment and Purification*, Springer International Publishing, Cham, (2014)

118. Chandrasekera, L.W.H.U., Pathiratne, A. and Pathiratne, K. Effects of water borne cadmium on biomarker enzymes and metallothioneins in Nile tilapia, *Oreochromis niloticus*. *J. Natl. Sci. Found. Sri Lanka*, **36**, 315 (2008)

119. Sabullah, M.K., Sulaiman, M.R., Shukor, M.Y.A., Syed, M.A., Shamaan, N.A., Khalid, A. and Ahmad, S.A. The Assessment of Cholinesterase from the Liver of Puntius Javanicus as Detection of Metal Ions . *Sci. World J.*, **2014**, 1–9 (2014)

120. Richetti, S.K., Rosemberg, D.B., Ventura-Lima, J., Monserrat, J.M., Bogo, M.R. and Bonan, C.D. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. *Neurotoxicology*, **32**, 116–122 (2011)

121. Chandrasekera, L.W.H.U., Pathiratne, A. and Pathiratne, K.A.S. Effects of water borne cadmium on biomarker enzymes and metallothioneins in Nile tilapia, *Oreochromis niloticus*. *J. Natl. Sci. Found. Sri Lanka*, **36**, 315–322 (2008)

122. Diaz de Cerio, O., Bilbao, E., Ruiz, P., Pardo, B.G., Martínez, P., Cajaraville, M.P. and Cancio, I. Hepatic gene transcription profiles in turbot (*Scophthalmus maximus*) experimentally exposed to heavy fuel oil n^{o} 6 and to styrene. *Mar. Environ. Res.*, **123**, 14–24 (2017)

123. Ahmad, I., Shukla, S., Kumar, A., Singh, B.K., Kumar, V., Chauhan, A.K., Singh, D., Pandey, H.P. and Singh, C. Biochemical and molecular mechanisms of N-acetyl cysteine and silymarin-mediated protection against maneb- and paraquat-induced hepatotoxicity in rats. *Chem. Biol. Interact.*, **201**, 9–18 (2013)

124. Harmon, S.M. Effects of Pollution on Freshwater Organisms *Water Environ. Res*., **80**, 1892–1917 (2008)

125. Assar, D.H., Mokhbatly, A.A.A., Ghazy, E.W., Ragab, A.E., Asa, S.A., Abdo, W., Elbialy, Z.I., Mohamed, N.E. and El-Far, A.H. Ameliorative effects of aspergillus awamori against the initiation of hepatocarcinogenesis induced by diethylnitrosamine in a rat model: Regulation of cyp19 and p53 gene expression *Antioxidants*, **10**, (6),922 (2021)

126. Yassine, T., Khalafalla, M.M., Mamdouh, M., Elbialy, Z.I., Salah, A.S., Ahmedou, A., Mamoon, A., El-Shehawi, A.M., Van Doan, H. and Dawood, M.A.O. The enhancement of the growth rate, intestinal health, expression of immune-related genes, and resistance against suboptimal water temperature in common carp (*Cyprinus carpio*) by dietary paraprobiotics. *Aquac. Reports*, **20**, 100729 (2021)

127. Fadl, S.E., Elbialy, Z.I., Abdo, W., Saad, A.H., Aboubakr, M., Abdeen, A., Elkamshishi, M.M., Salah, A.S., El-Mleeh, A., Almeer, R., Aleya, L., Abdel-Daim, M.M., Najda, A. and Abdelhiee, E.Y. Ameliorative effect of Spirulina and *Saccharomyces cerevisiae* against fipronil toxicity in *Oreochromis niloticus Ecotoxicol. Environ. Saf.*, **242**, 113899 (2022)

128. Fahmy, N., Abdelghafar, T., Omar, A., Abozeid, A.M., Brr, A., El-Midany, S.A. and Mohamed, R.A. Eco-Toxicological risk assessment of possible effects of potentially toxic heavy metals on water quality and performance of Nile tilapia (*Oreochromis niloticus*) in Burullus lake, North Delta, Egypt. *Egypt. J. Vet. Sci.*, 181–197 (2022)

129. Zeitoun, M.M., Sayed, E. and Mehana, E. Impact of Water Pollution with Heavy Metals on Fish Health: Overview and Updates *Glob. Vet.,* **12**, 219–231 (2014)

130. Deore, S. V and Wagh, S.B. Heavy metal induced histopathological alterations in liver of *Channa gachua* (Ham). *J. Exp. Sci.*, **3**, 35–38 (2012)

131. Abdel-Reheim, M.A., Ashour, A.A., Khattab, M.A. and Gaafar, A.G.A. *Quillaja saponaria* bark saponin attenuates methotrexate induced hepatic oxidative stress , inflammation and associated liver injury in rats *J. Appl. Pharm. Sci.*, **12**, 129–141 (2022)

132. Witeska, M. Stress in Fish-Hematological and Immunological Effects of Heavy Metals *Electron. J. Ichthyol.* **1**, 35–41 (2005)

133. Authman, M.M.N., Abbas, W.T. and Gaafar, A.Y. Metals concentrations in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) from illegal fish farm in Al-Minufiya Province, Egypt, and their effects on some tissues structures. *Ecotoxicol. Environ. Saf.*, **84**, 163–172 (2012)

134. Akan, J. Study on the Distribution of Heavy Metals in Different Tissues of Fishes from River Benue in Vinikilang, Adamawa State, Nigeria Br. *J. Appl. Sci. Technol.*, **2**, 311–333 (2012)

135. Doaa M. Mokhtar and Hanan H. Abd-Elhafeez. Histological Changes in Selected Organs of *Oreochromis niloticus* Exposed to Doses of Lead Acetate. *J. Life Sci. Biomed.*, **3**, 256–263 (2013)

قام *Quillaja saponaria* بتحسين أداء النمو ومكونات الدم ويحمي البلطي النيلي (*Oreochromis niloticus*) من تلف الأنسجة المؤكسدة والالتهابية الناجمة عن الرصاص والكادميوم

سمر توفيق[1] ، مالك محمد خلف الله[1] ، محمد ممدوح زايد[1] ، سلمي محمود زيد[2] و راضي علي محمد[1]

[1] قسم الإستزراع المائي - كلية الثروة السمكية والمصايد - جامعة كفر الشيخ – مصر.

[2] قسم علوم البحار- كلية العلوم - جامعة الإسكندرية – مصر.

الملخص

واحدة من أهم الوسائل الحديثة في الاستزراع السمكي الاهتمام برعاية الأسماك للحصول على منتج عالي الجودة. هدفت الدراسة الحالية إلى تقدير التأثير المتناقص لنبات (QS) *Quillaja saponaria* على أسماك البلطي النيلي المعرضة للمعادن الثقيلة وتأثيرها على أداء النمو والخصائص الدموية والكيميائية الحيوية والاستجابة التأكسدية وتلف الأنسجة. تم توزيع الأسماك (العدد = 90، متوسط الوزن: 61.74 ± 4.72 جم) بشكل عشوائي في ثلاث مكررات بمعدل 6 أسماك لكل حوض سمك 60 لتر. تم تعريض الأسماك لخليط الكادميوم (Cd) والرصاص (Pb) بجرعة 1.31 ملجم/لتر و58.4 ميكروجرام/لتر على التوالي وتم تلقي *QS*بجرعات 150، 300، 500 ملجم/كجم من علف الأسماك. أدى كل من Pd وCd إلى انخفاض ملحوظ في أداء النمو وتناول العلف كما أدت الى تدهور مؤشرات الدم والكيمياء الحيوية عن طريق انخفاض نسبة Hb وHTC وRBCs، وزيادة كرات الدم البيضاء، وزيادة مستويات ALT وAST وALP والبيليروبين واليوريا والدهون الثلاثية والجلوكوز والدهون الكلية وانخفاض البروتين الكلي والجلوبيولين والكوليسترول الجيد. تم تسجيل زيادة ملحوظة في مستويات MDA وانخفاض أنشطة SOD وGSH وChE% في الأسماك المعرضة للمعادن. كشف التحدي مع المعادن عن تأثير منتظم للتعبير الجيني لـ CYP19 في الكبد. عزز *Quillaja saponaria* أداء نمو الأسماك أثناء التعرض للمعادن. استعاد *Quillaja saponaria* مؤشرات الدم والكيمياء الحيوية الطبيعية عند مقارنتها بالمجموعة الضابطة. يعوق استيعاب *Quillaja saponaria* تأثير المعادن على إنزيمات الحالة التأكسدية (انخفاض قيم MDA وزيادة أنشطة SOD وGSH وChE%). حقق *Quillaja saponaria* توازن تكاثر الخلايا وموت الخلايا المبرمج للتعبيرات الجينية CYP19 وGPx. نستنتج من ذلك أن QS قد قلل التأثيرات السلبية للكادميوم والرصاص على الأسماك. عززت تركيزات 150 و 300 و 500 ملغم / كغم من علف الأسماك المحتوى على QS من أداء النمو والمؤشرات البيوكيميائية الدموية للأسماك المعرضة للمعادن الثقيلة. 150 ملجم QS/كجم علف للأسماك يعوق الإجهاد التأكسدي. كما حقق، وخاصة 500 ملجم QS/كجم، توازن الخلايا من التكاثر وموت الخلايا المبرمج.

الكلمات الدالة: سابونين - البلطي النيلي - الكادميوم - الرصاص.