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Ameliorative Impacts of Neem Leaf Powder on Cadmium Toxicity in Nile Tilapia: Growth Performance, Haemato-Biochemical, Oxidative and Pathological Insights

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Abstract

OUR research sought to clarify the impact of Neem Leaf Powder (NP) as a feed supplement in reducing the harmful effects of CdCl2 toxicity in Nile tilapia. Acclimated 360 fish were allocated randomly into 4 treatments with 3 repetitions in each group, including control group, CdCl2 intoxicated group (2 mg/L in water), and 2 groups fed on NP at 2 and 4 g/kg feed with exposure to 2 mg/L of CdCl2 for 60 days. Digestive enzymes, total protein, albumin, liver function indices (AST, ALT, and LDH), total cholesterol, and triglycerides, besides liver oxidant/antioxidant biomarkers (superoxide dismutase, catalase, glutathione peroxidase, glutathione S transferase) were measured using a spectrophotometer. Intestinal PCNA expression was detected using immunohistochemistry. Our data exhibited that tilapia exposed to water CdCl2 toxicity showed a significant decline in growth performance, feed utilization, fish survival, and intestinal digestive enzymes (protease, lipase, and amylase). Furthermore, CdCl2-intoxicated fish showed a substantial downregulation in hematological parameters, total protein, globulin, and some antioxidant indicators, including catalase, glutathione s-transferase, total and reduced glutathione, besides superoxide dismutase. Additionally, CdCl2 toxicity substantially upregulated albumin, glucose, liver enzymes, total cholesterol, triglycerides, glutathione peroxidase, and lipid peroxidation (LPO). The CdCl2 residues in the liver, gills, and muscles were significantly increased, particularly in hepatic tissues in the CdCl2-intoxicated group. Treatments with NP at both 2 and 4 g/kg diet impeded these deleterious effects by upregulating the growth parameters, hematological parameters, intestinal, hepatic, and antioxidant enzymes activities, with downregulation of LPO and lipid profile parameters with improvement in intestinal histoarchitecture.

Keywords: Neem Powder, tilapia, cadmium, growth performance.

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Introduction

Heavy metals from various sources, including industrial and anthropogenic activities, can significantly contaminate natural water systems [1]. The accumulation of heavy metals in aquatic animal tissues may reach to dangerous levels for people [2]. Cadmium (Cd) is a heavy metal with significant detrimental impacts on fish. Cadmium can harm the gills [3] and splenic tissue [4] of common carp. Nile tilapia can collect cadmium in their organs, resulting in histopathological changes in multiple tissues, especially the hepatic tissue, characterized by significant fatty change, hepatocellular necrosis, and vascular congestion. Furthermore, it causes significant histological changes in the intestine and pronounced glomerular atrophy and necrosis in the kidneys [5]. Toxicity of any toxicant arises when metabolic and detoxifying processes, along with excretory and storage activities, fail to mitigate absorption [6]. Cadmium is among the 126 contaminants identified by the US Environmental Protection Agency and is carcinogenic for multiple organs [7]. Also, by activating node-like receptor pyrine-3 (NLRP3) inflammasome, cadmium exposure in common carp produces pyroptosis of splenic lymphocytes [8].

In most tropical nations, a big evergreen tree *Azadirachta indica* (*A. indica*) with aromatic leaves and fruit that can be eaten, sometimes known as Margosa or neem, has possible therapeutic use due to its immunological, anti-inflammation, and anti-ulcer qualities [9, 10]. Furthermore, every component of neem tree has strong medicinal properties against several fungal, bacterial, and viral diseases as well as increasing antioxidant capacity [10, 11] and antidiabetic effect [12]. Likewise, some neem-based substances have been extensively utilized in aquariums for managing different fish parasites and fry predators during their culture in place of some hazardous pesticides or antibiotics. Although neem has been identified for its possible broad-spectrum preventive and therapeutic value, no studies on the diet supplemented with neem have been published to regulate Cd toxicity in tilapia. Thus, the aim of our work is to shed light on the possible beneficial effects of NP to mitigate water-born CdCl2 toxicity in *O. niloticus* via the upregulation of hematological markers, and antioxidant status and downregulation of inflammatory markers.

Material and Methods

Sources and collection of plant material

Freshly developed leaves of the neem tree were gathered from several sites in the Beni-Suef governorate. The gathered specimens were promptly enclosed in plastic bags. These specimens were kept at a temperature of 4 ◦C till the process of extraction. Then, it was rinsed with water and, afterward, with distilled water to eliminate dust. The specimens were dried in the shade at ambient temperature for three days. About 1000 grams of leaf samples were pulverized for 20 seconds using a grinding mill. The dried leaf specimens were ground into a powder. Neem leaf powders were extracted following the methodology outlined by Hossain et al. [13] .

Proximate analysis of Neem plant powder

Proximate Compositions

The proximate composition of the specimens, including moisture, fat, protein, ash, and crude fiber, was determined in duplicate using methods outlined by Onwuka [14]. Nitrogen content was assessed via the micro Kjeldahl method, and protein content was calculated by multiplying nitrogen values by 6.25 as described by Onwuka [14]. The total carbohydrate content was estimated by subtracting the sum of the other components from 100%. The proximate analysis yielded crude fiber, protein, and carbohydrate content values.

Vitamin A Estimation

Using a ceramic mortar, 1g sample was homogenized with 10 ml of distilled water and then filtered through filter paper. A 2 mL aliquot of the filtrate was shaken for 1 minute and heated at 60 °C for 20 minutes. After that, 2 mL of xylene was added, mixed thoroughly, and centrifuged for 10 minutes at 1500 rpm. The upper layer was gathered and relocated to a test tube. Before and after irradiating the extract for 30 minutes, the absorbance was estimated at 335 nm against xylene . [15]

Vitamin A concentration (Cx) was calculated using the following formula:

Cx = (A1 - A2) x 22.23, where A1 and A2 are the absorbance values before and after irradiation respectively.

Vitamin C Estimation

Using a ceramic mortar and pestle, 0.5 g of the specimen was mixed with 5 ml of 2% HCl and then filtered through filter paper. A 5 ml aliquot of the filtrate was moved to two conical flasks after it had been diluted to 100 mL. Iodole phenol dye was used to titrate the solution, and the titer value was noted. The result was calculated as (0.5 / 13.1) x titer value, which was then multiplied by 100 and divided by 5. The result was expressed in mg/100 mL [15].

Mineral Analysis

The specimen's mineral composition was analyzed by dry-ashing and spectroscopic methods. One g sample was ashed at 500 °C, then dissolved in HCl and analyzed by Atomic Absorption Spectrophotometer for mineral elements, flame photometry for sodium and potassium, and colorimetry (vanadomolybdate method, AOAC 975.03) for phosphorus [16].

Experimental fish and rearing

Diet formulation

Neem powder (NP): moisture (9.50 %), crude protein (1.50 %), crude lipids (2.20 %), crude fiber (5.90 %), ash content (2.80 %), and carbohydrate (78.10 %); Crude protein (CP); Vitamin premix: each kg contains vitamin A (8000000 IU), vitamin D3 (2,000,000 IU), vitamin E (7000 mg), vitamin K3 (1500 mg), biotin (50 mg), pantothenic acid (7000 mg), folic acid (700 mg), nicotinic (20,000 mg), vitamin B1 (700 mg), vitamin B2 (3500 mg), vitamin B6 (1000 mg), vitamin B12 (7 mg); Mineral premix: each kg contains copper sulfate (2.7 g), iron sulfate (20 g), zinc sulfate (4.0 g), manganese sulfate (5.3 g), sodium selenite (70 mg), calcium iodine (0.34 g), cobalt sulfate (70 mg), and calcium phosphate dibasic dihydrate up to 1 kg (Table 1) [17].

Experimental design

Based on preliminary studies and laboratory facilities, 360 Tilapia Oreochromis niloticus (weight 45 ± 2 gram) were purchased from Aquaculture farms of Kafr El-Sheik governorate, Egypt, and were transferred to the laboratory at Alexandria University. Fish were acclimatized for two weeks and provided with a ration purchased from Alar Aqua feed company 3 times/day (3% of the fish weight). The physicochemical conditions of the water in all tanks were similar (water temperature 28 ± 2 ˚C, 6 ml/l dissolved Oxygen, 8.3 pH, and Ammonia 0.03 ± 0.01 ml/l). After acclimatization time, 360 fish were selected randomly and were divided into 4 treatments with 3 repetitions (30 fish in each repetition). Control group: without CdCl2 treatment in water. Cdcl2 group: 2 mg/L of CdCl2 in water [18]. 2.0 NP group: Basal diet + 2.0 g/kg feed of NP + 2 mg/L of CdCl2 [19]. 4.0 NP group: Basal diet + 4.0 g/kg feed of NP + 2 mg/L of CdCl2 [9] .

After completion of the study, individual fish weights were recorded to assess feed efficiency and growth performance using pre-established formulas [20-23].

The calculated parameters included:

Weight gain (WG): final body weight (FBW) - initial body weight (IBW)

Feed intake: total feed offered / total number of fish

Feed conversion ratio (FCR): FI / (FBW - IBW)

Survival rate: (final number of fish / initial number of fish) × 100

Hepatosomatic index (HSI): (liver weight/body weight) × 100

Relative intestinal length (RIL): Intestine length (mm) / body mass (g)

Blood and tissue collection

After 60 days, fish were restricted from food for a whole day and anesthetized with 0.02% benzocaine solution to minimize stress. Three randomly chosen fish from each aquarium (9 fish/treatment) were sampled, and two blood specimens were taken from the caudal vein via sterile syringes and aliquoted into 2 test tubes: one with heparin for hematological analysis and another without anticoagulant, which was permitted to clot at room temperature for 30 minutes. The tube with the clotted sample was then centrifuged at 1500 × g for 15 minutes for serum separation. Then, samples of serum were kept at -20°C for subsequent biochemical analysis.

Hepatic and intestinal tissue specimens were gathered after the desiccation of the fish. For the histoarchitecture analysis, these tissue samples were reserved in 10% neutral buffered formalin solution. Hepatic homogenate was prepared by homogenization of the tissue specimens. For additional examination, the supernatants were then gathered in a cool Eppendorf and stored at -80°C.

Digestive enzyme analysis

To study the impact of the NP on digestive enzyme activities (protease, lipase, and amylase), desecration of three randomly chosen fish from each aquarium (9 fish/treatment) were sampled under aseptic circumstances, and their intestinal tissue was gathered and washed with cold distilled water. The intestinal contents were then homogenized in phosphate-buffered saline (PBS) at 4°C. The homogenate was then centrifuged at 5000g for 20 minutes at 4°C. The homogenate supernatants were placed at 4°C for analysis of digestive enzymes, including protease, lipase, and amylase by using a commercial kit (Sigma-Aldrich) and a spectrophotometer as directed by the instructions of the manufacturer [24].

Hematological Investigation

Hematological parameters, including red and white blood cell counts (RBCs and WBCs) and differential leukocytic counts, were estimated [25]. The packed cell volume (PCV %) was evaluated as described by Stoskopf [26]. While hemoglobin (Hb) level was estimated using commercial colorimetric cyanmethemoglobin kits rendering to van Kampen and Zijlstra [27].

Serum biochemical analysis

Serum biochemical parameters were analyzed following the manufacturer's instructions for kits from Spinreact® Co., Spain. Colorimetric analysis was used to assess both total protein and albumin levels [28]. While subtracting albumin from total protein levels to calculate the globulin levels [29]. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured calorimetrically using Reitman and Frankel's method [30]. Lactate dehydrogenase (LDH) activity was

assessed using Bergmeyer and Bernt method [31]. Additionally, serum levels of total cholesterol and triglycerides (TG) were evaluated spectrophotometrically based on Allain, Poon [32] and Fossati and Prencipe [33], respectively. Additionally, serum glucose levels were assessed utilizing Trinder [34] method, respectively.

Liver Oxidant/Antioxidant Stress Biomarkers

Hepatic superoxide dismutase (SOD) activity was assessed using technique at 560 nm [35]. Catalase (CAT) was detected by estimating the resultant quinonimine dye at 510 nm [36]. Also, the activity of glutathione peroxidase (GSH-Px) was assessed by methyl catechol reaction at 340 nm as described by Paglia and Valentine [37]. Additionally, the activity of glutathione S transferase (GST) was assessed by following the rise in absorbance at 340 nm [38]. The reduced glutathione (GSH) concentration was measured according to the methods recorded by Beutler, Duron [39]. Total glutathione (TG), including the reduced (GSH) and all oxidized disulfide forms as oxidized glutathione (GSSG), were measured according to the method of Griffith [40]. Malondialdehyde (MDA) is considered a lipid peroxidation (LPO) indicator and was measured spectrophotometrically Ohkawa, Ohishi [41] using the thiobarbituric acid at 532 nm. As directed by the manufacturer, all parameters were measured spectrophotometrically using certain commercial diagnostic kits from Bio diagnostic Co., Giza, Egypt.

Estimation of cadmium in tissues

Tissue specimens (liver, gill, and muscle) were collected from each group, and cadmium (Cd) levels were measured according to Begum, Mustafa [42]. [43]Results were stated as micrograms per gram of moist tissue.

Histopathological studies

Formalin-fixed hepatic and intestinal specimens were dehydrated in a graded alcohol series. Then, xylene clearing was performed, then routine paraffin embedding. The tissues were cut into 5 µm sections and stained with hematoxylin and eosin (H&E) and Alcian blue/Periodic acid Schiff [44].

Immunohistochemical protein assay

Slides underwent deparaffinization and dehydration, then immersion in a 10 mM sodium citrate buffer and transferred to a microwave for antigen retrieval. Then, 3% hydrogen peroxide was applied for 30 minutes to block endogenous peroxidase and to hinder non-specific binding. For sixty minutes, 10% normal blocking serum was applied at room temperature. Rat monoclonal antibodies were used to incubate the slides against PCNA (Catalog # (F-2): sc-25280, 1:50 dilution, Santa Cruz). After that, a biotinylated secondary antibody and horseradish peroxidase reagent were applied for 30 minutes at 37°C. Following every step, the slides underwent 3 times of rinsing with phosphate-buffered saline. Then, slides were incubated with 3,3'-diaminobenzidine tetrahydrochloride reagent for 3 min. The slides were then washed with distilled water, counterstained with Mayer's hematoxylin, and mounted with DPX. The control negative slides have been created by eliminating the primary antibody incubating stage [45]. The percent of brown-positive immune-stained areas were quantified using Fiji image analyzer in 10 randomly selected fields for each fish [46, 47].

Real-time PCR analysis of hepatic Immune and antioxidant-related genes

Following the procedure outlined by Chomczynski [48], 100 mg of total RNA was extracted from the hepatic specimen using the TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA). RNA quantity was quantified using a nanodrop spectrophotometer (Quawell, USA). Subsequently, cDNA was generated utilizing the High-Capacity cDNA Reverse Transcription Kits (Fermentas, Waltham, MA, USA), adhering to the manufacturer's guidelines. The extracted RNA and cDNA were thereafter preserved at −80 °C until required for application. The mRNA transcripts for immune-related genes as Colony-stimulating factor 1 receptor (CSF1R), Immunoglobulin heavy chain (IgMH) interferon 1 alpha-like (INF1α), and antioxidant genes as catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) were investigated by real-time PCR (ABI PRISM 7500) using SYBR Green PCR Core Reagents. Also, we detected mRNA expressions of inflammatory genes such as Il-8, Il-10, and HSP70. The elongation factor 1 α (EF1α) and β-actin functioned as the reference (housekeeping) gene with primer set (Table 2). The mRNA expression computation was derived from the Ct values collected, employing the $2^{-\Delta\Delta CT}$ approach [49].

Statistical analysis

The homogeneity of data variance and normality were assessed using Levene's test and the Kolmogorov-Smirnov test, respectively, with GraphPad Prism software (version 10.5). One-way ANOVA was employed to analyze the data, and significant differences between treated groups were estimated using Tukey's test ($P < 0.05$) [50]. Villar necrosis was evaluated using the Kruskal-Wallis test followed by Dunn's test. Statistical findings are presented as means ± standard error of the mean (means ± SEM) [51].

Results

Phytochemical analysis

As depicted in Table 3, phytochemical analysis of NP showed the highest concentrations of tannin, trypsin inhibitor, total carotenoids, and oxalate.

Quantitative phytochemicals of NP present are total ash content, protein, fat, fiber, carbohydrates, vitamin A and vitamin C. Mineral constituents of fresh leaves of Azadirachta indica powder showed the result after 1st, 2nd and third 3 readings of calcium, sodium, and phosphorus with the highest concentration of potassium (Table 4). As shown in Tables 5 and 6, in the preliminary phytochemical investigations of ethanolic extract of the Azadirachta indica, it was observed that the quantitative phytochemical components were carotenes, carotenoids, oxalate, phytate, terpenoids, trypsin inhibitor, saponins, total phenol, alkaloids, xanthophyll, and flavonoids.

Growth performance indices, feed efficiency, and survival rate

The growth performance indices, feed utilization and survivability of O. niloticus were significantly influenced ($P < 0.05$) by Cd toxicity and NP exposure (Fig. 1) for a 60-day trial. The results showed that CdCl2 intoxication resulted in a noticeable drop in final weight ($P<0.05$), weight gain besides feed intake as compared to control fish. At the same time, these growth parameters showed a considerable increment ($P < 0.05$) in treatment groups relative to the CdCl2 intoxicated one with highest increment in 4.0 NP treated group.

Regarding FCR, CdCl2 intoxication resulted in a significant increment ($P < 0.05$) in comparison to the control fish while substantially decreased ($P < 0.05$) in treatment groups in contrast to the CdCl2 intoxicated group with the lowest FCR in 4.0 NP treated group. Also, CdCl2 intoxication significantly decreased ($P < 0.05$) fish survival rate (%) relative to the control group and this decrement was substantially upregulated ($P < 0.05$) in treatment groups with the highest survival rate (%) in the 4.0 NP treated group (Fig. 1).

Hepatosomatic and intestinal index

As shown in Fig. 1, HIS and RIL displayed a noteworthy downregulation ($P < 0.05$) in the fish intoxicated with CdCl2 by 25.98% and 48.57%, respectively if compared with the control group. The dietary supplementation of NP at different concentrations to intoxicated fish exhibited a significant upregulation ($P < 0.05$) in the abovementioned parameters by 60.63% and 77.7%, respectively in 2.0 NP treated group and by 75.53% and 100% in 4.0 NP treated group when compared with the Cd-intoxicated group. The most improvements were observed in 4.0 NP treated group.

Intestinal digestive enzyme activities

According to Fig. 2, intestinal digestive enzymes (protease, lipase, and amylase) revealed a considerable decline ($P < 0.05$) in waterborne CdCl2 exposed fish by 69.15%, 39.66% and 39.12%, respectively in contrast to the control group. While, following neem exposure, protease and amylase activities indicated a substantial rise ($P < 0.05$) 57.98% and 49.28% in 2.0 NP group and by 122.9% and 92.91% in 4.0 NP treated group relative to Cd exposed fish. Also, the lipase activity showed non-significant change in 2.0 NP treated group and significant raise in 4.0 NP treated group by 40.92% relative to CdCl2 exposed fish.

Hematological findings

As shown in Fig. 3, the CdCl2 intoxicated fish revealed a substantial drop ($P < 0.05$) in RBCs, WBCs counts, Hb, PCV %, neutrophils and lymphocytic % without any significant ($P > 0.05$) change in monocyte % relative to the control fish. While the treatments with NP at different concentrations showed significant increments ($P < 0.05$) in the aforementioned parameters relative to CdCl2-intoxicated fish.

Serum biochemical analysis

According to biochemical parameters in Fig.4, the fish intoxicated with CdCl2 displayed a considerable decline ($P < 0.05$) in serum total protein and globulin with a substantial increase in liver enzyme activities (AST, ALT and LDH), glucose, total cholesterol, triglyceride levels compared to the control group. While the dietary supplementation with NP at two concentrations to intoxicated fish showed significant biochemical improvement as noticed a remarkable increase ($P < 0.05$) in serum levels of total protein and globulin with a substantial decrease ($P < 0.05$) in other abovementioned parameters if compared with the CdCl2-intoxicated group.

Hepatic Oxidative and antioxidant markers

As presented in Fig.5, the intoxication of drinking water of fish with CdCl2 resulted in substantial decrements ($P < 0.05$) in hepatic SOD, CAT, GST activities, TG and GSH levels with an observable increment ($P < 0.05$) in LPO (MDA) level and GSH-PX activity when contrasted with the control group. While the addition of NP at two concentrations in the feed of CdCl2-intoxicated fish showed a significant increase ($P < 0.05$) in the activities of the aforementioned antioxidant enzymes with a substantial decrease ($P < 0.05$) in LPO level and GSH-PX if compared with the CdCl2-intoxicated group.

CdCl2 residue in fish organs

The CdCl2 residue in the liver, gills and muscles were remarkably increased ($P < 0.05$) in the CdCl2 intoxicated fish by 11.35%, 4fold and 414%, respectively relative to the control one with more substantial ($P < 0.05$) increment in liver then gills then muscular tissue. Meanwhile, tested organs of treatment groups presented a considerable decline ($P < 0.05$) by 44.93%, 25.56% and 61.71% in 2.0 NP

treated group and 51.34%, 54.09% and 74.28% in 4.0 NP treated group relative to the CdCl2 intoxicated group with a considerably lowest concentration in 4.0 NP treated group (Fig.6).

Histopathological investigation

Histopathological examination of control group intestinal tissue revealed normal histoarchitecture of intestinal villi lined by simple columnar epithelium, submucosal glands, tunica muscularis and serosa (Fig. 7A). Conversely, the CdCl2 group showed villar necrosis with intense mononuclear inflammatory infiltrates that destroy the submucosal glands (Fig. 7B). The addition of NP at different concentrations in the feed of CdCl2-intoxicated fish showed dose-dependent improvement of intestinal histoarchitecture (Fig. 7C-D). Semi-quantitative analysis of the intestinal necrosis score showed a noteworthy increase ($P < 0.05$) in CdCl2 treated group relative to control group. While addition of 2.0 NP showed a non-significant change in Villar necrosis score but 4.0 NP displayed a substantial downregulation ($P < 0.05$) in villar necrosis score when compared with the CdCl2-intoxicated group (Fig. 7E).

AB/PAS stain showed intense blue goblet cells staining in control group (Fig. 8A) while CdCl2 group exhibited week AB stained goblet cells (Fig. 8B). While, addition of NP at different concentrations showed moderate to mild blue goblet cells staining. (Fig. 8C-D). Semi-quantitative analysis of the goblet cell area% score showed a noteworthy decrease ($P < 0.05$) in CdCl2 treated group relative to control group. While addition of 2.0 NP and 4.0 NP displayed a substantial upregulation ($P < 0.05$) in goblet cell area% score when compared with the CdCl2-intoxicated group (Fig. 8E).

Regarding to immunohistochemical examination of PCNA, intestinal tissue of O. niloticus in control group showed an intense PCNA immunoreactivity (Fig. 9A) while, CdCl2 group exhibited weak and mild PCNA immunoreactivity (Fig. 9B). While addition of NP at different concentrations showed mild to moderate PCNA immunoreaction (Fig. 9C-D). Semi-quantitative analysis of PCNA area% in CdCl2 group revealed a significant downregulation ($P < 0.05$) in PCNA immunoexpression as compared to control group. While the addition of NP at different concentrations presented a considerable increment ($P < 0.05$) in PCNA area% if compared with the CdCl2-intoxicated group with pronounced decline in G4 (Fig. 9E).

Effect of Neem Leave powder (NP) against cadmium toxicity on the expression of immune and antioxidant-related genes

As presented in Fig. 10A-E, the intoxication of drinking water of fish with CdCl2 resulted in substantial decrements ($P < 0.05$) in the expression of immune-related genes (CSF1R, IgMH and INF1α) by 12.93%, 34.05% and 36.08%, respectively with a significant upregulation ($P < 0.05$) in HSP70 by 31.20% relative to the control group. The addition of NP at different concentrations in the feed of Cd-intoxicated fish showed noticeable increments ($P < 0.05$) in the mRNA expressions of CSF1R (20.14% and 47.24%, respectively) and INF1α (12.87% and 31.13%, respectively) and downregulation in HSP70 (15.6% and 17.83%, respectively) compared with the Cd-intoxicated group. Additionally, gene expression of Ig MH in 2.0 NP showed no discernible difference ($P > 0.05$) relative to Cd-intoxicated fish, while 4.0 NP group showed a significant improvement in IgMH mRNA expression by 35.59% relative to the Cd-intoxicated group.

As shown in Fig. 10F-H, SOD and CAT mRNA expressions demonstrated a noteworthy downregulation ($P < 0.05$) by 45.09% and 40.19%, respectively, while mRNA expressions of GR showed a substantial upregulation ($P < 0.05$) by 19.29% in the Cd-intoxicated group relative to the control group. Whereas the addition of NP at different concentrations in the feed of Cd-intoxicated fish displayed non-significant change in 2.0 NP group and a noteworthy increase ($P < 0.05$) in 4.0 NP group of SOD and CAT expressions with a substantial downregulation ($P < 0.05$) in GR expression by 12.47% and 20.09% if compared with the Cd-intoxicated fish. The most improvements were observed in 4.0 NP group.

Discussion

The growth performance is a vital index that signifies the health and developmental status of fish [52]. In our investigation, CdCl2 intoxication in O. niloticus resulted in a significant decline in growth, feed utilization, survival rate, and hepatosomatic index with an increment in FCR. Additionally, the exposure of O. niloticus to CdCl2 caused a significant decrease in the digestive enzyme's activities of (protease, amylase and lipase) in comparison with control. These findings could be attributed to the presence of Cd in fish farming water, resulting in a decline in fish feed consumption, resulting in a reduction of growth rate, and body weight. Also, exposure to Cd may provoke inflammation in fish viscera and hepatopancreas. Consequently, there is a notable decline in the hepatosomatic index and intestinal length of fish [53]. Lastly, Cd may provoke significant enteritis and diminish the activity of digestive enzymes which is the hallmark of digestive efficiency [54], with subsequent impeding the absorption and utilization of feed by fish [55]. These results parallel previous studies [56, 57]. Furthermore, Hani, Turies [58] demonstrated a substantial decline in the digestive enzymes activities of three-spined stickleback (Gasterosteus aculeatus) after long-term exposure to

Cd. This may be returned firstly to the ability of Cd to bind with thiol groups specifically cysteine residues in the active centers of digestive enzymes, leading to inhibition of both lipase and amylase activities [59]. Secondly, Cd stress can induce intestinal oxidative stress leading to intestinal necrosis, disturbances in both antioxidant and immune systems, and inhibition of the activities of digestive enzymes [57, 60]. Also, Lucia et al.[61] reported the role of Cd to hinder the synthesis of fatty acid reducing the lipase activity.

The addition of NP at different concentrations in the feed of CdCl2-intoxicated fish showed a significant increase in growth indices, feed utilization, survival rate, and hepatosomatic index with a decline in FCR. These observations are in line with these reported by Talpur and Ikhwanuddin [9] who reported that Azadirachta indica (neem) leaf-supplemented diets efficiently restored growth rate and feed utilization with enhancement in survival rate for Vibrio harveyi-infected Asian seabass. Another trial conducted by Abdel Rahman, Amer [62] reported that neem seed protein hydrolysate effectively restored the loss of feed intake and consequently body weight in Aeromonas veronii-infected O. niloticus. Also, the increments in the activities of digestive enzymes after neem supplementation at different concentrations increased indicating its role in improving intestinal performance. This result accords with Radwan et al. [19] who reported the role of ethanolic neem extract (1 and 2g/kg diet for 60 days) to increase the activities of lipase, amylase, and chymotrypsin in Nile tilapia juveniles relative to control fish. Also, Abdel Rahman, Amer [63] displayed that administration of neem seed protein hydrolysate for two months (10, 20, 30 and 40 g/kg diet) significantly increased lipase and amylase enzyme activity dependently on the dose. This could be explained by the capability of neem to enhance the bacteria in the intestine to improve its secretory action to digestive enzymes and facilitating the nutrient absorption[9, 63]. Also, da Silva [64] returned the improved digestion and absorption in the neem group to its role in exciting the release of digestive enzymes.

Hematological examination is one of the tools commonly used to evaluate the health and welfare of fish, and to assess the influence of environmental factors on fish [65]. An accurate fish hemogram analysis is associated with a substantial elevation in RBCs, Hb, and hematocrit level (Ht) [53]. The current study reveals that the exposure of O. niloticus to CdCl2 resulted in a substantial reduction in RBCs, Hb and Ht, WBCs, neutrophils, and lymphocytes % relative to the control.

These outcomes are consistent with the outcomes of Abdel-Tawwab, Khalil [53] who stated a substantial reduction in RBCs count, Hb and Ht, WBCs count in O. niloticus fish exposed to Cd Cl2 (2 mg/L water) for 60 days. This reduction might be due to the inhibition of erythrocyte production resulting from the reduction of haemosynthesis or hemolytic crisis that leads to the destruction of mature RBCs leading to severe anemia in fish after exposure to various contaminants as cd [66]. Or could be returned to the abnormalities in the hemopoietic organ and the metabolic status associated with fish intoxicated with Cd [53, 67]. On the other hand, the leukopenia induced by Cd associated with a decrease in the neutrophilic and lymphocytic % may be attributed to a general stress response induced by toxicity or immunosuppression caused by Cd's toxic action on circulating leukocytes [68]. On the other hand, the treatment with different concentrations of NP showed an improvement the general health of fish by inducing significant increases in RBCs, Hb, Ht levels, neutrophilic and lymphocytic % in comparative to those intoxicated with Cd. These results agree with Radwan, Manaa [19] who found that the dietary treatment of Nile tilapia juveniles with aqueous and ethanolic extract of neem at 1 and 2g/kg feed for 60 days increased RBCs, WBCs, Hb, and Ht levels compared to control fish. They returned these results that neem can improve the synthesis of erythropoietin, increasing the synthesis of RBCs and inducing the capacity of blood to transport oxygen to tissues. Consistently, the increment of WBCs and lymphocytes play various roles in the immunity as triggering the lymphoid tissues to regenerate lymphoid follicles and promoting the maturation of T lymphocytes in lymphoid organs [69, 70]. Other results of Oniovosa, Aina [71] disagree with our results as they found that dietary treatment of catfish with neem extract at 3.5 and 7% LC50 for 4 weeks decreased RBCs, WBCs, Hb and Ht and increased both neutrophils and lymphocytes. This disagreement might be returned to different species, dose and environmental conditions.

The colony-stimulating factor 1 receptor (CSF1R), often referred to as the macrophage colony-stimulating factor receptor (M-CSFR), is essential for the proliferation, survival, and migration of macrophages [72]. Also, INF-1α is crucial for the presentation of antigen and the suppression of virus replication [73]. Our results showed that CdCl2 intoxication resulted in a decline in immune-related genes such as CSF1R, IgMH, and INF1α and an increase in HSP70 mRNA transcript. They found that the immune-related enzymes of C. auratus indigentiaus were suppressed by high Cd concentrations. This reduction may lead to damaging the immune function and consequently increase vulnerability to infection [74]. These results indicate the role of long-term exposure to Cd Cl2 in suppressing innate immunity leading to harmful effects on the health of fish. Also, Xia, Ding [75] reported a significant decrease in IgM gene expression and consequently, a decrease its level in

serum in the Cd-exposed group (1.0 mg/L) of C. auratus indigentiaus). Furthermore, a study by Jabeen, Ishaq [76] showed higher levels of metallothionein and HSP70 gene expression after sublethal Cd exposure to grass carp juveniles for 28 days. The addition of neem at different concentrations to Cd-exposed fish improved the immunity in a manner that is depending on dosage. In line with those findings, neem supplementation with high doses of powder resulted in an upregulation in immune-related genes such as CSF1R, IgMH and INF1-α and downregulation in HSP70 mRNA transcript. These consequences might result from the bioactive substances in neem that stimulate and boost cellular and humoral mediated pathways and induce antibody production [77]. Additionally, neem leaf contains terpenoids that have powerful antioxidative effect improving overall health and immunologic functions [78]. Additionally, it is approved that plants with immunostimulant effects can adjust and enhance the immune gene expression [79].

When investigating the impact of metal contamination on fish health, ecotoxicological investigations may employ serum biochemical parameters as sensitive biomarkers. Our results showed that exposure of O. niloticus to Cd caused a significant increase in the serum AST, ALT, and LDH activities, glucose, total cholesterol, and triglyceride levels. These results come in accordance with Heydarnejad, Khosravian-Hemamai [80] who found that exposure of rainbow trout to the sub-lethal concentrations of Cd (3 μg/l) for 30 days increased TP, AST, ALT, total cholesterol and triglyceride except for glucose which appears without changes in comparison with control. Also, Abdel-Tawwab, Khalil [53] demonstrated a substantial increment in AST, ALT, LDH activities, glucose, triglyceride and total cholesterol levels in gilthead seabream (Sparus aurata L.) exposed to waterborne Cd toxicity (1 and 2.0 mg Cd/L) for 60 days. According to the proteinogram, our results presented a noteworthy decrement in both TP and globulin with a significant increment in albumin level. This agrees with Kori-Siakpere, Ake [81] who recorded that the exposure of Clarias graiepinus to Cd (0.5 and 1 mg Cd/ l) showed a substantial decline in the level of protein and Kumar [82] who recorded a significant reduction in protein levels in Clarias batrachus exposed to 1 mg of CdSO4 for 32 days. Also, Al-Asgah, Abdel-Warith [83] reported a substantial reduction in serum TP of O. niloticus exposed to CdCl2 at 10%, 20% and 30% of the LC50 for 10, 20 and 30 days. This decrement may be due to the decrease the protein synthesis due to liver damage [84]. Or increase the loss of protein due to renal damage and decrease the resorption [85]. While disagreeing with Elarabany, Bahnsawy [86] who reported that the exposure of African catfish to sub-lethal doses of Cd (10% and 30% LC50) increased TP level without changes in albumin in comparison with control. This hyperproteinemia may be due to kidney damage [87]. The hyperalbuminemia recorded in our study in O. niloticus exposed to Cd can be explained by increasing the albumin synthesis in the liver in response to Cd exposure to protect against metal-induced damage as reported by Fırat and Kargın [88]. Also, Fabisiak, Sedlov [89]stated that the redox modification of albumin modulates its physiological function and acts as an oxidative stress biomarker. Halliwell and Gutteridge [89] suggested that binding metal with albumin in an inactive form prevents the degradation of peroxides and prevents the production of hydroxyl radicals so, concluded the role of albumin as an antioxidant.

The metabolism of proteins and amino acids depends deeply on ALT and AST. The liver damage induced by Cd leads to an increase in the cell membrane permeability and increases the release into the bloodstream [90, 91]. In addition, chronic stress may cause various physiological changes in glucose levels as hyperglycemia [92]. So, the elevation of glucose levels in our study may be due to stimulation of glycogenolysis which means the release of glucose from glycogen stored in muscles and the liver by stress hormones as cortisol [93]. While the increment in triglycerides and total cholesterol levels may be attributed to the inhibitory effect of Cd on the thyroid gland functions as it is reported that Cd causes hypothyroidism that is associated with hypertriglyceridemia or may be due to liver dysfunction leading to disturbance of lipid metabolism [94].

While, the addition of different concentrations of NP in the feed of fish improved the previous biochemical parameters as they displayed a substantial increase in serum TP and globulin with a substantial decline in liver enzymes, glucose, triglyceride and total cholesterol levels. These come in agreement with Adamu, Aliyu-Paiko [11] that found decrease in the serum levels of triglycerides, cholesterol, and glucose either significantly or insignificantly in African catfish after treatment with different doses of Azadirachta indica Leaf powder (0.192, 0.096, 0.048 mg/L). They returned this decrement to that the fish can utilize different sources of energy to produce its metabolic energy in the presence of the leaf powder as the fish utilized the lipid as a source of metabolism. Also, the decrease in serum triglyceride may be due to increase the lipolysis rate during the exposure period to the plant [95]. Also, Yarmohammadi, Mehri [96] explained the hypolipidemic and the hypoglycemic effects of neem by its role in inducing the antioxidant system and inhibiting oxidative stress. Also, Radwan, Manaa [19] reported the role of ethanolic extract of neem at 1 and 2g/g diet to increase the serum total protein and globulin with decrease in the serum activities of AST and ALT in Nile tilapia

juveniles if compared with the control. Also, agree with Abdel Rahman, Amer [62] who proved the modulatory hepatic functional effect of dietary neem extract. This may be returned to the ability of neem to improve the hepatic functional status [97] leading to an increase the protein synthesis due to containing several bioactive components as rutin, quercetin, and azadirachtin- that decreases hepatocellular necrosis and restoring its normal function [77].

Concerning oxidative and antioxidant markers, the exposure of fish to CdCl2 in water led to a noteworthy increment in the LPO and GSH-Px associated with a significant decrement in the antioxidant system if compared with the control fish. These results align with previously published findings by Al-Asgah, Abdel-Warith [83] who returned these changes to the accumulation of Cd in the liver after long-term exposure of O. niloticus to Cd. As, the liver is thought to be the primary organ where Cd accumulates and is crucial to its metabolism and transformation in many fish species.

The accumulation of cd in the liver may lead to various hepatocellular changes and damage due to the Cd ability to bond with disulfide, sulfhydryl, and amine groups of many cellular components such as proteins, nucleus, mitochondrial membranes, and lysosomes leading to homeostasis disruption and induction of oxidative stress [98]. Also, El-Sokkary, Nafady [99] and Arroyo, Flores [100] reported that Cd can stimulate oxidative stress, causing increased lipid peroxidation causing membrane and protein damage. The antioxidant defense system involves both enzymatic as SOD, GPx, GST, CAT and non-enzymatic antioxidants (GSH) which protect tissue against the damaging effects of metal [101]. This antioxidant system plays an important role in scavenging ROS. According to the present finding, the total hepatic antioxidant level significantly decreased in Cd-exposed O. niloticus fish. This decrement may be due to disruption in the synthesis of both enzymatic and non-enzymatic antioxidants that make the fish more susceptible to oxidative stress and cellular damage as reported by a Drąg-Kozak, Pawlica-Gosiewska [102] in the hepatopancreas of Prussian carp (Carassius gibelio Bloch). Also, Liu, Deng [103] recorded a substantial increase in SOD and CAT in the hepatopancreas of Macrobrachium nipponensis after cd exposure in water. Also, Zhang, Xie [104] found a significant decrease in the hepatic gene expressions related to CAT, SOD and GST in O. niloticus exposed to waterborne Cd (2mg/L) leading to decrease their activities. In our study, the Cd led to an increase in hepatic GSH-PX similar to Messaoudi, Barhoumi [105] that reported an increment in the GSH-PX activity in marine fish (Salaria basilisca) after 14 days of exposure to Cd. They considered this increment to be an indication of a good response to counterbalance the damaging oxidative stress induced by Cd accumulation. The upregulation of GSH-PX may be attributed to the Cd toxic excitant effect, leading to the development of an overcompensation mechanism that induces the upregulation of antioxidant expression to facilitate the elimination of ROS in vivo mitigating the oxidative damage [106]. Also, Lee, Choi [107] showed upregulation of GSH-PX levels in the gills of olive flounder (Paralichthys olivaceus) after exposure to cd stress to eliminate the produced ROS. In contrast, addition of neem at different concentrations alone or in combination significantly increased the hepatic aforementioned enzymes activities and total glutathione levels with a substantial reduction in LPO levels and GSH-PX activities relative to Cd-exposed fish. The increments are greater in the groups with the highest dose and the combined ones. These findings indicated the role of neem in improving antioxidant activity and decreasing LPO. These antioxidant effects are associated to the active components of neem as flavonoids and phenolics that enhance the antioxidative status, reducing the LPO and decreasing the production of free radicals [79, 108]. It was discovered that flavonoids play an antioxidant role by shielding healthy cells from damage caused by free radicals[109]. Abdel Rahman, Amer [63] demonstrated that the high content of antioxidants in A. indica extract can prevent LPO and improve the body's antioxidant status. The decrement of GSH-PX may be returned to its consumption to overcome the oxidative stress. These results come in accordance with Radwan, Manaa [19], who reported the role of ethanolic neem extract (1 and 2g/kg diet) to decrease the hepatic MDA levels and increasing the SOD and CAT activities in juveniles of Nile tilapia relative to the control. Likewise, Ibrahim, Elshopakey [108] reported a noteworthy reduction in serum MDA level with a notable increase in GSH level and CAT activity after treatment of bacterial infected O niloticus fish with chitosan neem nanocapsule (1mg/l). Also, Abu-Elala, Khattab [110] reported that the addition of neem leaf powder for 2 weeks can induce a substantial decline in hepatic MDA with increment of GSH level in lead intoxicated O. niloticus fish.

In the present investigation Cd residue in the liver, gills and muscles was considerably higher in the group of fish intoxicated with CdCl2 than in the control group with a higher accumulation liver then gills but muscular tissue had the lowest levels. Furthermore, the findings of the current investigation align with the data presented by Filipović Marijić and Raspor [111], who stated that prolonged exposure to metals resulted in their accumulation in storage organs such as the hepatic and renal tissue. Research conducted on many freshwater fish species has demonstrated that the liver is the primary organ for metal accumulation and significantly contributes to the preservation, redistribution, elimination, or transformation of metals [112]. According to our

study, Al-Asgah, Abdel-Warith [83] discovered that the muscular tissue of O. niloticus had the lowest quantities of metals after fish exposure than the gills and liver. While. the addition of neem powder at different concentrations to Cd-exposed fish showed a considerable decline in Cd residue as compared to the CdCl2 intoxicated group. Sharma and Bhattacharyya [113] showed that neem leaf powder had a significant promise for use as an adsorbent for Cd in aqueous solutions.

As far as we are aware, our research is the first to show the impact of neem extract powder against Cd bioaccumulation in various tissues. Building on the observed benefits of neem powder supplementation at 4 g/kg in mitigating CdCl2 toxicity in fish, future studies should explore the long-term effects of this dietary intervention on various fish species under different environmental conditions. Investigations into more molecular and immunological mechanisms by which neem confers protection could provide deeper insights into its therapeutic potential. Additionally, optimizing the dosage and formulation (e.g., encapsulation, fermented forms) may enhance its bioavailability and effectiveness. Comparative studies with other phytogenic additives may also help validate neem as a cost-effective and sustainable solution for aquaculture. Finally, field-scale trials in commercial fish farms are recommended to assess the practicality, scalability, and economic feasibility of incorporating neem powder into standard feeding protocols.

Conclusion

Waterborne CdCl2 toxicity significantly negatively influenced the growth performance, digestive in O. niloticus. Thus, the reduction of antioxidant responses and immune biomarkers in Nile tilapia may function as a biomarker for heavy metal contamination as cadmium as presents in our study. This discovery provides significant insights for monitoring of water contamination. Additionally, our study's results indicated that incorporating neem leaf powder in specified quantities into fish diets could enhance their heme synthesis, immune responses, and anti-oxidant status So, it is recommended to use neem powder especially at 4 g/kg in the diet of fish to enhance the general all health condition of fish, reducing mortality rates following Cd toxicity and so increase its economic benefits.

Acknowledgments

Not applicable.

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This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The research was approved by the institutional animal care and use committee (IACUC), Alexanderia University under approval number (ALEXU- IACUC, 013-2024-12-01/ 0307011).

TABLE 1. Ingredients and chemical analysis of the formulated experimental diets

Ingredients	Formulated diet groups		
	Control	**NP 2%**	**NP 4%**
Yellow corn	23.50 %	21.50 %	19.50 %
Soybean meal (46% CP)	26.00 %	26.00 %	26.00 %
Fish meal (65% CP)	20.00 %	20.00 %	20.00 %
Corn gluten meal (60% CP)	8.50 %	8.50 %	8.50 %
Wheat bran	10.00 %	10.00 %	10.00 %
Corn oil	3.00 %	3.00 %	3.00 %
Cod liver oil	2.00 %	2.00 %	2.00 %
Starch	4.00 %	4.00 %	4.00 %
Vitamin premix	1.00 %	1.00 %	1.00 %
Mineral premix	2.00 %	2.00 %	2.00 %
Neem powder	0 %	2 %	4.00 %
Total percentages	100 %	100 %	100 %
Chemical analysis of formulated diets			
Crude protein	32.88%	32.73 %	32.58 %
Crude fat	8.99 %	8.99 %	8.97 %
Crude fiber	3.40 %	3.49 %	3.56 %
Digestible energy	4135 kcal/kg	4095 kcal/kg	4055 kcal/kg

TABLE 2. Primer design used for gene expression in the study

Gene name	Primer sequence (5′→3′)	Reference
Elongation factor 1- alpha	F: AGAACGTCTCCGTCAAGGAA R: TGATGACCTGAGCGTTGAAG	[114]
Colony-stimulating factor 1 receptor (*CSF1R*)	F: CAAGCTCATTTCAACGGTCA R: CGAAGGAAGTTCAGCAGGTC	[114]
Immunoglobulin heavy chain (*IgMH*)	F: GCAAAGGGATGATGCTGTCT R: GAGGTCAATGCGGTTTTTGT	[114]
Interferon 1 alpha-like (INF1*α*)	F: ATGGGAGGAGAACACAGTGG R: TGTCGTATTGCTGTGGCTTC	[114]
Catalase (CAT)	F: ATGAGGAGGAGCGACAGAGA R: AATTCTCGACCATGCGTTTC	[114]
Super oxide dismutase (SOD)	F: GGAGGTGAACCACAAGGAGA R: TACAGCCACCGTAACAGCAG	[114]
Glutathione reductase (GR)	F: TCTGCACGATCATGGTGATT R: TGCGATTTAGGTGACTGACG	[114]
Il-10	F: 5′-CTGCTAGATCAGTCCGTCGAA-3′ R: 5′-GCAGAACCGTGTCCAGGTAA-3′	[115]
HSP70	F:5′-CATCGCCTACGGTCTGGACAA-3′ R:5′-TGCCGTCTTCAATGGTCAGGAT-3′	[116]
β-actin	F: 5′-AGCAAGCAGGAGTACGATGAG-3′ R: 5′-TGTGTGGTGTGTGGTTGTTTTG-3′	[117]

TABLE 3. Phytochemical constituent of powder from the leaves of neem samples

Phytochemical	Result
Tannin (mg/kg)	987.0
Oxalate (mg/kg)	89.4
Phytate (mg/kg)	9.3
Terpenoids (mg/kg)	20.4
Trypsin inhibitor (mg/kg)	109.5
Total phenol (mg/kg)	22.4
Total Carotenoids (g/kg)	102.58
Total Carotenes (g/kg)	82.7
Xanthophyll (g/kg)	28.6
Flavonoids (g/kg)	72. 3
Saponins (%)	7.8

TABLE 4. Proximate composition and mineral constituents of fresh leaves of neem

Parameters	Result		
	1st reading	**2nd reading**	**3rd reading**
Moisture %	9.50	9.55	9.61
Ash content %	2.87	2.82	2.92
Protein %	1.52	1.55	1.44
Fat %	2.12	2.09	2.16
Fibre %	5.95	5.91	5.84
Carbohydrate %	78.09	78.13	78.03
Total	100	100	100
Vitamin A (mg/100 g)	175	189	168
Vitamin C (mg/100 g)	262	248	277
Potasium (mg/100 g)	229.24	235.63	231.74
Calcium(mg/100 g)	162.18	170.05	159.49
Sodium(mg/100 g)	169.17	180.65	163.67
Phosphorus (mg/100 g)	44.21	39.34	47.29

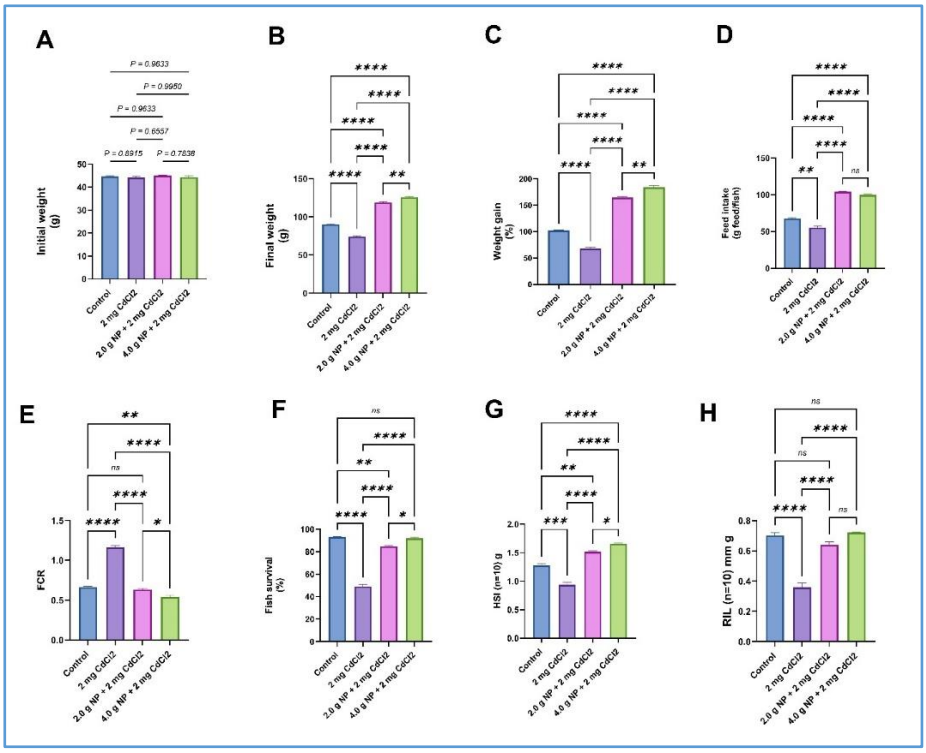

Fig. 1. Effects of different levels of dietary Neem Leave powder (NP) and waterborne cadmium chloride (CdCl2) toxicity on growth parameters, feed utilization and survival rate of Nile tilapia (*O. niloticus*) fed on experimental diets during 60 days. (A) Initial weight (g). (B) Final weight (g). (C) Weight gain (%). (D) feed intake (g). (E) Feed conversion ratio (FCR). (F) Fish survival (%). (G) Hepatosomatic index (HSI, g). (H) Relative intestinal length (RIL, mmg). Data presented as mean± standard error (MSE). Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

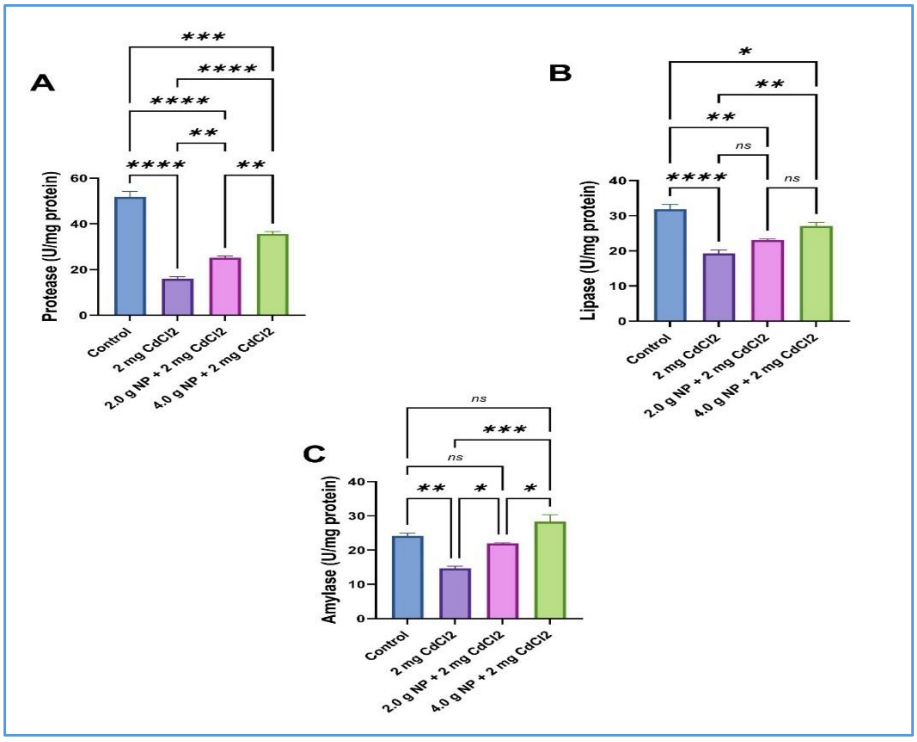

Fig. 2. Effects of different levels of dietary Neem Leave powder (NP) against waterborne cadmium toxicity on intestinal digestive enzymes activities of Nile tilapia (O. niloticus) fed on experimental diets during 60 days. (A) Protease (U/mg protein). (B) Lipase (U/mg protein). (C) Amylase (U/mg protein). Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

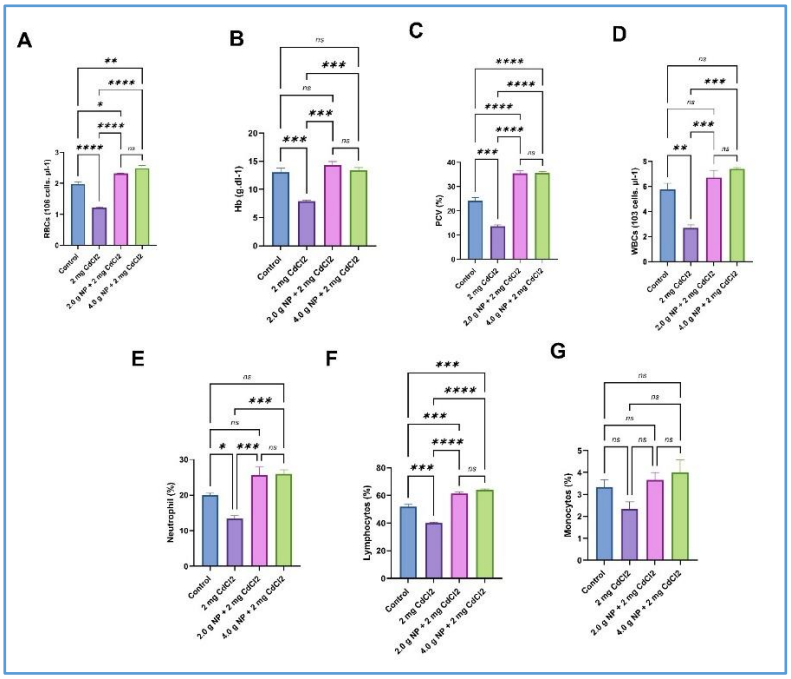

Fig. 3. Effects of different levels of dietary Neem Leave powder (NP) against waterborne cadmium chloride (CdCl2) toxicity on the hematological parameters of Nile tilapia (*O. niloticus*) fed on experimental diets during 60 days. (A) Red blood cells (RBCs). (B) Hemoglobin (Hb). (C) Packed cell volume (PCV). (D) White blood cells count (WBCs). (E) Neutrophil (%). (F) Lymphocyte (%). (G) Monocyte (%).Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****:**

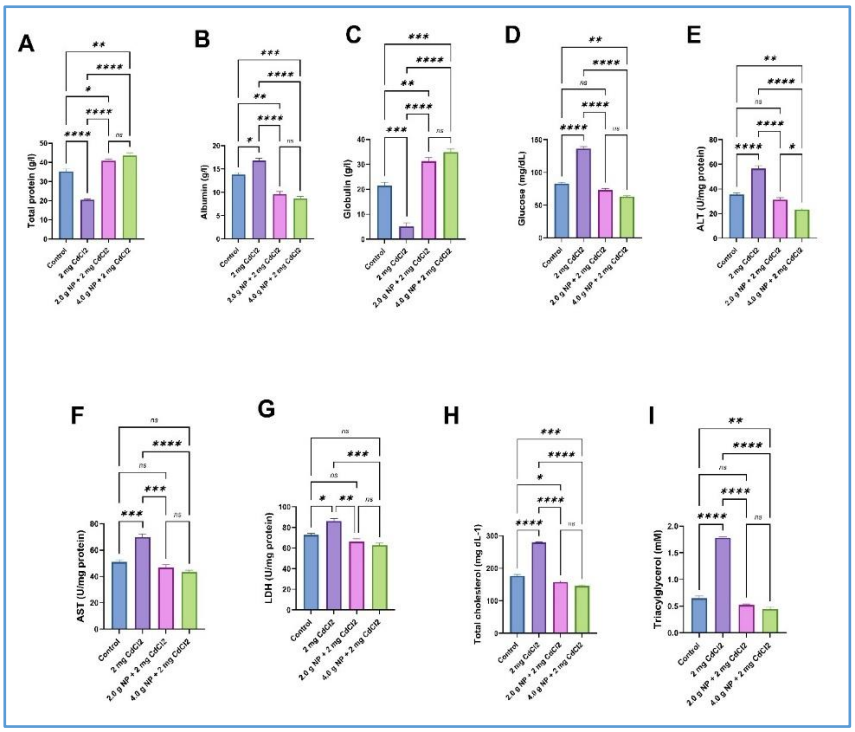

Fig. 4. Effects of different levels of dietary supplementation of Neem Leave powder (NP) against waterborne cadmium chloride (CdCl2) toxicity on serum biochemical parameters of Nile tilapia (*O. niloticus*) fed on experimental diets during 60 days. (A) Total protein (g/l). (B) Albumin (g/l). (C) Globulin (g/l). (D) Glucose (mg/dl). (E) Alanine aminotransferase (ALT, U/mg). (F) Aspartate aminotransferase (AST, U/mg). (G) Lactate dehydrogenase (LDH, U/mg). (H) Total cholesterol (mg/dl). (I) Triglyceride (mM). Data presented as mean± standard error (MSE). Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

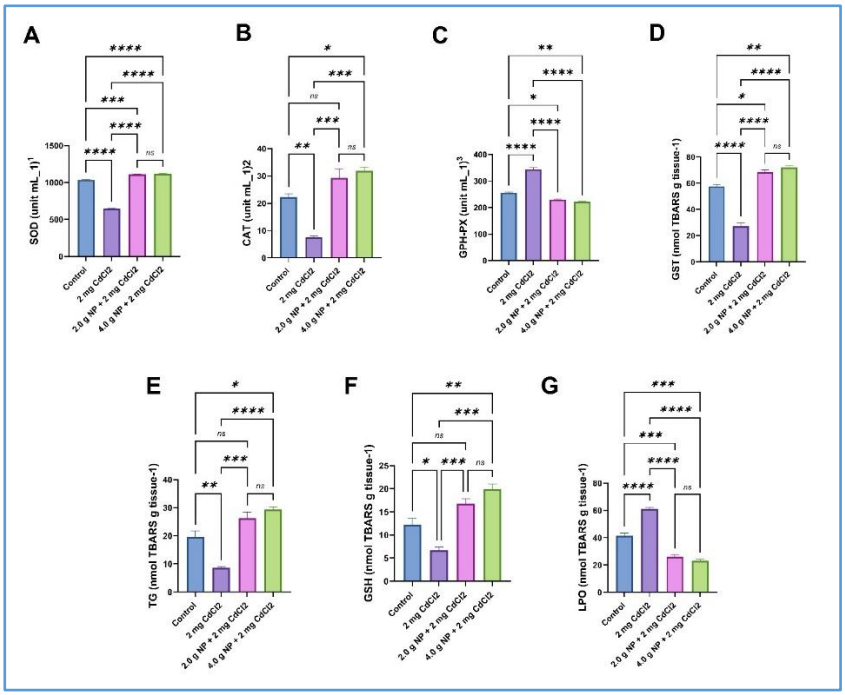

Fig.5 Effects of different levels of dietary Neem Leave powder (NP) against waterborne cadmium toxicity on antioxidant indicators in liver tissues of Nile tilapia (*O. niloticus*) fed on experimental diets during 60 days. (A) Superoxide dismutase (SOD). (B) Catalase (CAT). (C) Glutathione peroxidase (GSH-Px). (D) Glutathione s-transferase (GST). (E) Total Glutathione (TG). (F) Reduced Glutathione (GSH). (G) Lipid Peroxidation (LPO). Data presented as mean± standard error (MSE). Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

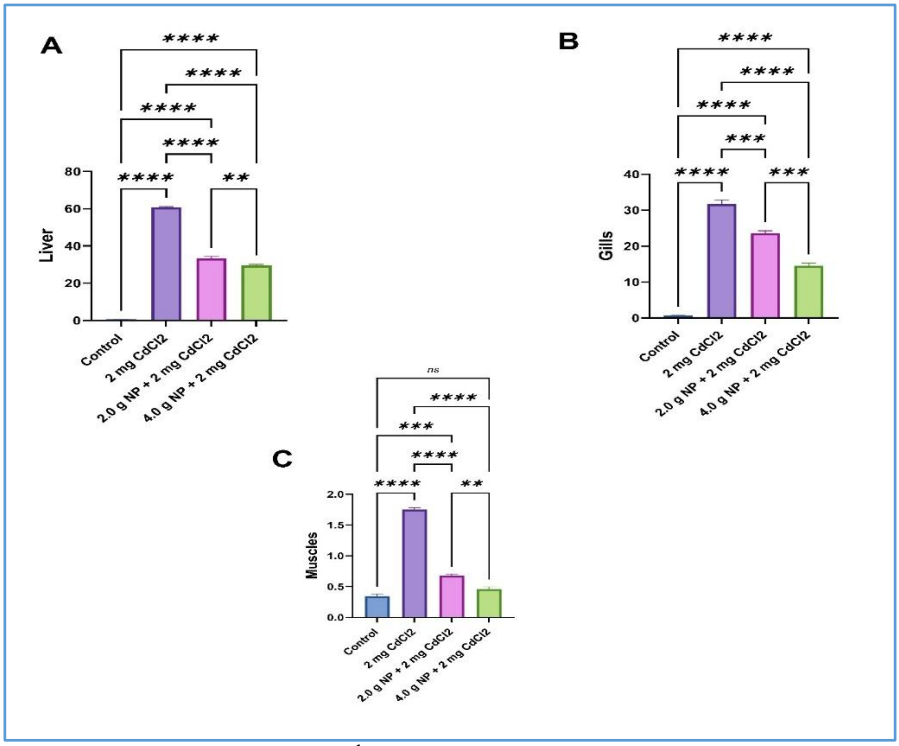

Fig.6. Cadmium residues (mg kg^{-1} wet weight) in liver, gills and muscles of Nile tilapia, *O. niloticus*, experimentally exposed to 2.5 mg Cd L^{-1} and feeding on different levels of Neem Leave powder (NP) -supplemented diet for 60 days. (A) Liver. (B) Gills. (C) Muscles. Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

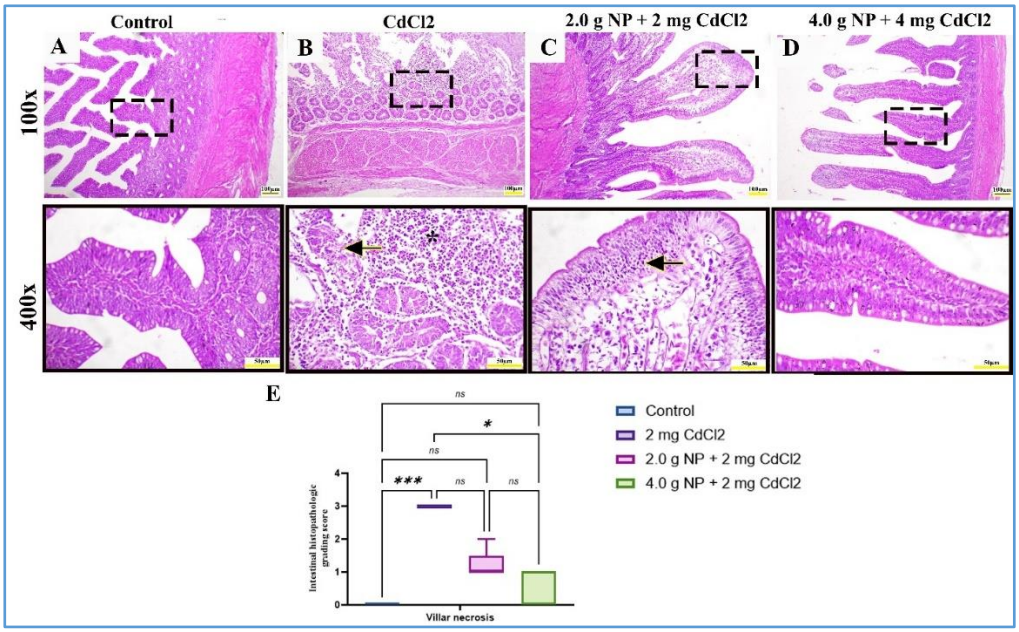

Fig. 7. Effects of different levels of dietary neem Leave powder (NP) against waterborne cadmium chloride (CdCl2) toxicity on hepatopancreatic histopathological alterations of Nile tilapia (*O. niloticus*) fed on experimental diets for 60 days. (A) Control group showing typical intestinal villar histoarchitecture, submucosal glands, tunica muscularis and serosa. (B) CdCl2 group showing villar necrosis and sloughing besides necrosis of submucosal glands (arrow) with intense inflammatory infiltrates. (C) 2.0 g/kg feed of NP + CdCl2 group showing moderate preservation for villar histoarchitecture. (D) 4.0 g/kg feed of NP + CdCl2 group showing congestion and dilatation of villar vessels with intraepithelial lymphocytic invasion (arrow). (I) Intestinal histopathological grading score. H & E. Bar (A-D)=100 µm. Bar (inset)=50 µm. Score was analyzed by Kruskal–Walli's test followed by Dunn's post-hock test. Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

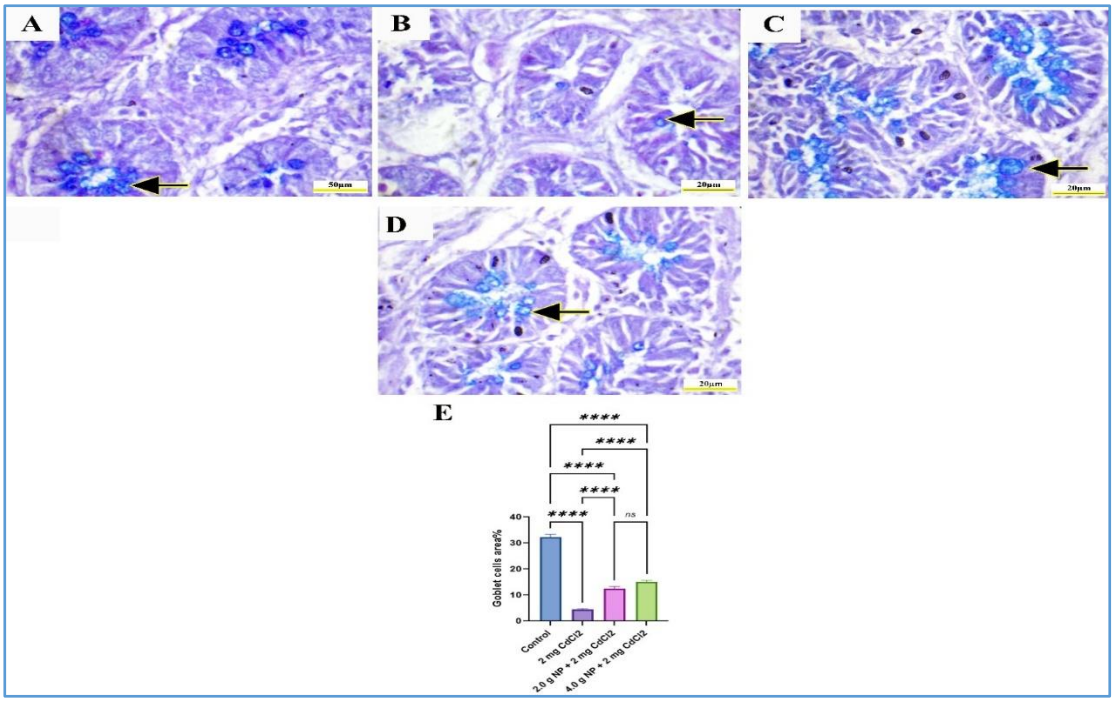

Fig.8. Effects of different levels of dietary neem Leave powder (NP) against waterborne cadmium chloride (CdCl2) toxicity on hepatopancreatic histopathological alterations of Nile tilapia (*O. niloticus*) fed on experimental diets for 60 days. (A) Control group. (B) CdCl2 group. (C) 2.0 g/kg feed of NP + 2 mg/L of CdCl2 treated group. (D) 4.0 g/kg feed of NP + 2 mg/L of CdCl2 treated group. Alcian blue/Periodic acid Schiff stain. Arrows indicate Alcian blue positive goblet cells. Bar=20 µm. Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

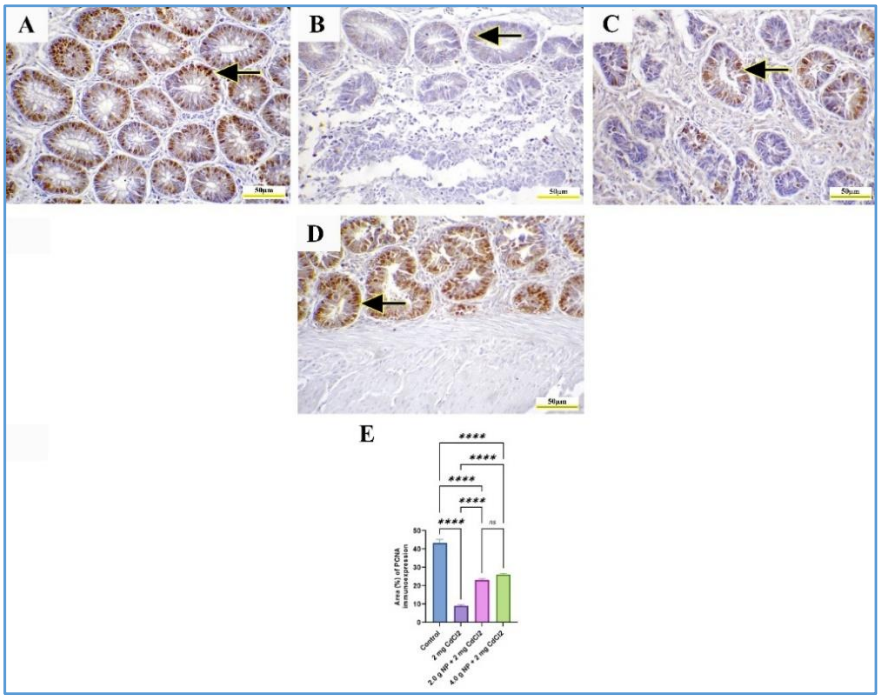

Fig. 9. Effects of different levels of dietary neem Leave powder (NP) against waterborne cadmium (CdCl2) toxicity on hepatopancreatic histopathological alterations of Nile tilapia (*O. niloticus*) fed on experimental diets for 60 days. (A) Control group. (B) CdCl2 group. (C) 2.0 g/kg feed of NP + 2 mg/L of CdCl2 treated group. (D) 4.0 g/kg feed of NP + 2 mg/L of CdCl2 treated group. PCNA (Proliferating cell nuclear antigen) immunostaining. Arrows indicate brown-positive nuclear immunoreactivity for PCNA. Bar=50 µm. Data presented as mean± standard error (SEM). Data were analyzed by one Way ANOVA followed by Tukey's test for multiple comparisons. Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

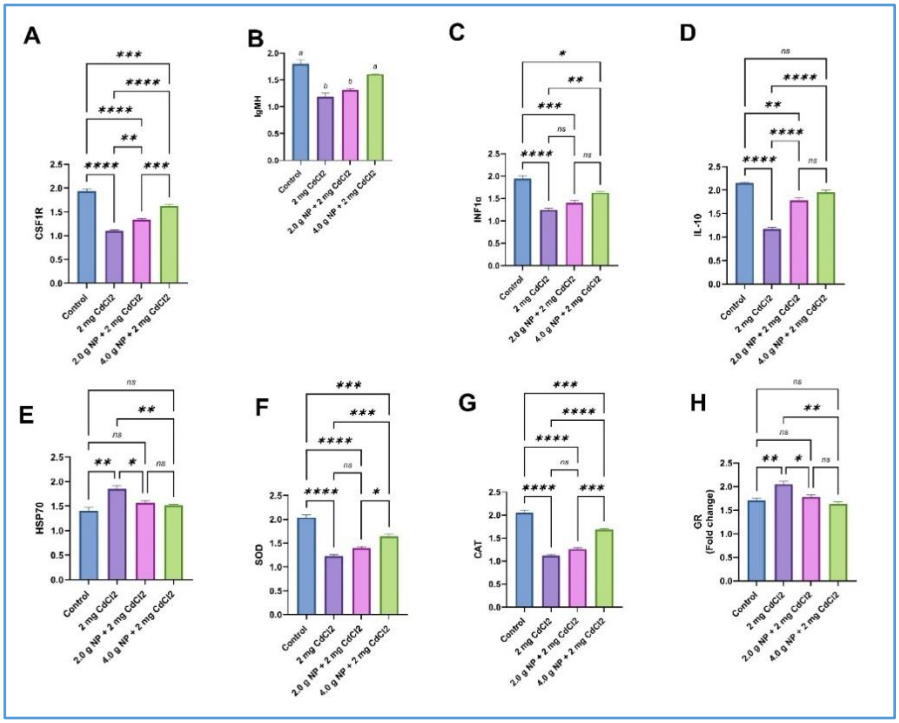

Fig.10. Effects of different levels of dietary Neem Leave powder (NP) against waterborne cadmium toxicity on relative gene expression of immune, inflammatory and antioxidant-related genes in liver of Nile tilapia (*O. niloticus*) fed on experimental diets during 60 days. (A) Colony-stimulating factor 1 receptor (CSF1R) mRNA transcript. (B) Immunoglobulin heavy chain (IgMH) mRNA transcript. (C) Interferon 1 alpha-like (INF1α) mRNA transcript. (D) Interleukin 10 (IL-10) mRNA transcript. (E) Heat shock protein 70 (HSP70) mRNA transcript. (F) Super oxide dismutase (SOD) mRNA transcript. (G) Catalase (CAT) mRNA transcript. (H) Glutathione reductase (GR) mRNA transcript. Statistical significance indicated by *: <0.05%; ** : <0.01%, *: <0.001%, ****: <0.0001%.**

References

1. Vélez, D. and Montoro, R., Arsenic speciation in manufactured seafood products. *Journal of Food Protection*, **61**(9), 1240-1245 (1998).
2. Kalay, M. and Erdem, C., Effect of cadmium accumulation on total protein levels in Tilapia nilotica. *Turkish Journal of Veterinary & Animal Sciences*, **27**(6), 1367-1374 (2003).
3. Chen, J., Chen, D., Li, J., Liu, Y., Gu, X. and Teng, X., Cadmium-induced oxidative stress and immunosuppression mediated mitochondrial apoptosis via JNK-FoxO3a-PUMA pathway in common carp (Cyprinus carpio L.) gills. *Aquatic Toxicology*, **233** 105775 (2021).
4. Chen, J., Xu, Y., Han, Q., Yao, Y., Xing, H. and Teng, X., Immunosuppression, oxidative stress, and glycometabolism disorder caused by cadmium in common carp (Cyprinus carpio L.): application of transcriptome analysis in risk assessment of environmental contaminant cadmium. *Journal Of Hazardous Materials*, **366**, 386-394 (2019).
5. Omer, S.A., Elobeid, M.A., Fouad, D., Daghestani, M.H., Al-Olayan, E.M., Elamin, M.H., Virk, P. and El-Mahassna, A., Cadmium bioaccumulation and toxicity in tilapia fish (Oreochromis niloticus). *Journal of Animal and Veterinary Advances*, **11**(10), 1601-1606 (2012).
6. Jarup, L., Hazards of heavy metal contamination. *Br. Med. Bull*, **68**, 167–182(2003).
7. Waalkes, M.P., Cadmium carcinogenesis in review. *Journal of Inorganic Biochemistry*, **79**(1-4), 241-244 (2000).
8. Zhang, Y., Liu, Q., Yin, H. and Li, S., Cadmium exposure induces pyroptosis of lymphocytes in carp pronephros and spleens by activating NLRP3. *Ecotoxicology and Environmental Safety*, **202**, 110903 (2020).
9. Talpur, A.D. and Ikhwanuddin, M., Azadirachta indica (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, Lates calcarifer challenged with Vibrio harveyi. *Fish & Shellfish Immunology*, **34**(1), 254-264 (2013).
10. Shah, A.S., Gunjal, M.A. and Juvekar, A.R., Immunomostimulatory activity of aqueous extract of Azadirachta indica flowers on specific and non specific immune response. *Journal of Natural Remedies*, 35-42 (2009).
11. Adamu, K.M., Aliyu-Paiko, M., Abdullahi, F. and Mustapha A., Effects of Azadirachta indica Leaf Powder on some Biochemical Parameters of the African catfish (Clarias gariepinus). *Nigerian Journal of Basic and Applied Sciences*, **25**(2), 41-50 (2017).
12. Chattopadhyay, R.R., Possible mechanism of antihyperglycemic effect of Azadirachta indica leaf extract. Part IV. *General Pharmacology*, **27**(3), 431-434 (1996).
13. Hossain, M.A., Al-Toubi, W.A.S., Weli, A.M., Al-Riyami, Q.A. and Al-Sabahi, J.N., Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. *Journal of Taibah University for Science*, **7**(4), 181-188 (2013).
14. Onwuka, G.I., Food analysis and instrumentation: theory and practice. 2005: Napthali prints.
15. Rutkowski, M. and Grzegorczyk, K., Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. *Acta Scientiarum Polonorum Technologia Alimentaria*, **6**(3), 17-28 (2007).
16. Chemists A.O.O.A., Official Methods of Analysis: Changes in Official Methods of Analysis Made at the Annual Meeting. Supplement. Vol. 15. 1990.
17. Council N.R., Nutrent requirement of fish. Committee on Animal Nutrition. Board on Agriculture. National Academy of Sciences. National Academy Press. Washington DC., USA, 114 (1993).
18. Ibrahim, I.H., Said, N.M., Abd-allah, M.M., Ramadan A.A. and Abdel-karim, E.A., Potential Effects of Cadmium Chloride and Glyphosate on DNA Damage in Nile tilapia. *Biochemistry Letters*, **14**(1), 313-328 (2018).
19. Radwan, M., Manaa, E.A., El-Feky, M.M., Mohammadein, A., Al Malki, J.S., Badawy, L.A. and Abbas, M.M.M., Elucidating the effect of dietary neem (Azadirachta indica) on growth performance, haemato-biochemical, immunonological response, and anti-pathogenic capacity of Nile tilapia juveniles. *Veterinary Research Communications*, **48**(6), 3621-3638 (2024).
20. Mohammadi, G., Rafiee, G., El Basuini, M.F., Van Doan, H., Ahmed, H.A., Dawood, M.A. and Abdel-Latif, H.M., Oregano (Origanum vulgare), St John's-wort (Hypericum perforatum), and lemon balm (Melissa officinalis) extracts improved the growth rate, antioxidative, and immunological responses in Nile tilapia (Oreochromis niloticus) infected with Aeromonas hydrophila. *Aquaculture Reports*, **18**, 100445 (2020).
21. Abdelghany, M.F., El-Sawy, H.B., Abd El-hameed, S.A., Khames, M.K., Abdel-Latif, H.M., and Naiel, M.A., Effects of dietary Nannochloropsis oculata on growth performance, serum biochemical parameters, immune responses, and resistance against Aeromonas veronii challenge in Nile tilapia (Oreochromis niloticus). *Fish & Shellfish Immunology*, **107**, 277-288 (2020).
22. Ming, J.-H., Ye, J.-Y., Zhang, Y.-X., Xu, P. and Xie, J., Effects of dietary reduced glutathione on growth performance, non-specific immunity, antioxidant capacity and expression levels of IGF-I and HSP70 mRNA of grass carp (Ctenopharyngodon idella). *Aquaculture*, **438**, 39-46 (2015).
23. Kumar, V., Wang, H.-P., Lalgudi, R.S., Mcgraw, B., Cain, R. and Rosentrater, K.A., Processed soybean meal as an alternative protein source for yellow perch (Perca flavescens) feed. *Aquaculture Nutrition*, **25**(4), 917-931 (2019).

24. Wang, Y.-B., Effect of probiotics on growth performance and digestive enzyme activity of the shrimp Penaeus vannamei. *Aquaculture*, **269**(1), 259-264 (2007).

25. Bain, B.J., Bates, I., Laffan, M.A. and Lewis, S.M., Dacie and Lewis practical haematology: expert consult: online and print. 2016: Elsevier Health Sciences.

26. Stoskopf, M., Fish medicine WB Saunders Company. Harcourt Brace Jovanovick Inc. The Curtis centre, independence square, west Philadelphia, Pennyslavania, 19106, 214-216 (1993).

27. Van Kampen, E. and Zijlstra, W., Standardization of hemoglobinometry II. The hemiglobincyanide method. *Clinica Chimica Acta*, **6**(4), 538-544 (1961).

28. Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, Jr T. and Schaffer, R., A candidate reference method for determination of total protein in serum. I. Development and validation. *Clinical Chemistry*, **27**(10), 1642-1650 (1981).

29. Coles, E., Plasma proteins. Veterinary clinical pathology, 2nd ed., WB Saunders Co., Philadelphia, Pennsylvania, USA, 558-560 (1974).

30. Reitman, S. and Frankel, S., A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal Of Clinical Pathology*, **28**(1), 56-63 (1957).

31. Bergrneyer, H. and Bernt, E., Lactate dehydrogenase-UV-assay with pyruvate and NADH. Methods of enzymatic analysis (Bergmeyer HU, ed) vol, **2**, 574-579 (1974).

32. Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., and Fu, P.C., Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, **20**(4), 470-475 (1974).

33. Fossati, P. and Prencipe, L., Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, **28**(10), 2077-2080 (1982).

34. Trinder, P., Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals Of Clinical Biochemistry*, **6**(1), 24-27 (1969).

35. Nishikimi, M., Rao, N.A. and Yagi, K., The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical And Biophysical Research Communications*, **46**(2), 849-854 (1972).

36. Aebi, H., Catalase in vitro, in Methods in enzymology. 1984, Elsevier. p. 121-126.

37. Paglia, D.E. and Valentine, W.N., Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal Of Laboratory And Clinical Medicine*, **70**(1), 158-169 (1967).

38. Habig, W.H., Pabst, M.J. and Jakoby, W.B., Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, **249**(22), 7130-7139 (1974).

39. Beutler, E., Duron, O. and Kelly, B.M., Improved method for determination of blood glutathione. (1963).

40. Griffith, O.W., Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical Biochemistry*, **106**(1), 207-212 (1980).

41. Ohkawa, H., Ohishi, N. and Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, **95**(2), 351-358 (1979).

42. Begum, A., Mustafa, A.I., Amin, M.N., Chowdhury, T.R., Quraishi, S.B. and Banu, N., Levels of heavy metals in tissues of shingi fish (Heteropneustes fossilis) from Buriganga River, Bangladesh. *Environmental Monitoring and Assessment*, **185,** 5461-5469 (2013).

43. Officer P.Z.A., Certificate of Analysis–Certified Reference Material.

44. Suvarna, K.S., Layton, C. and Bancroft, J.D., Bancroft's theory and practice of histological techniques E-Book. 2018: Elsevier health sciences.

45. Jackson, P. and Blythe, D., Immunohistochemical techniques. *Theory and Practice Of Histological Techniques*, **7**, 381-426 (2008).

46. Crowe, A.R. and Yue, W., Semi-quantitative determination of protein expression using immunohistochemistry staining and analysis: an integrated protocol. *Bio-Protocol.*, **9**(24), e3465-e3465 (2019).

47. Vis, A.N., Kranse, R., Nigg, A.L. and Van Der Kwast, T.H., Quantitative analysis of the decay of immunoreactivity in stored prostate needle biopsy sections. *American Journal of Clinical Pathology*, **113**(3), 369-373 (2000).

48. Chomczynski, P., A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *Biotechniques*, **15**(3), 532-536 (1993).

49. Schmittgen, T.D. and Livak, K.J., Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, **3**(6), 1101-1108 (2008).

50. Dytham, C., Choosing and using statistics: a biologist's guide. 2011: John Wiley & Sons.

51. Festing, M.F. and Altman, D.G., Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR Journal*, **43**(4), 244-258 (2002).

52. Zhang, Q., Xie, Y., Zhang, Y., Huang, E., Meng, L., Liu, Y. and Tong, T., Effects of dietary supplementation with chitosan on the muscle composition, digestion, lipid metabolism, and stress resistance of Juvenile Tilapia (Oreochromis niloticus) exposed to cadmium-induced Stress. *Animals*, **14**(4), 541 (2024).

53. Abdel-Tawwab, M., Khalil, R.H., Younis, N.A., Abo Selema, T.A., Saad, A.H., El-Werwary, S.O., Gouda, A.H., Soliman, A.M., Shady, S.H. and Monier M.N., Saccharomyces cerevisiae supplemented diets mitigate the effects of waterborne cadmium toxicity on gilthead seabream (Sparus aurata L.): growth performance, haemato-biochemical, stress biomarkers, and histopathological investigations. *Veterinary Research Communications*, **48**(1), 69-84 (2024).

54. Zaki, M.A.A., Khalil, H.S., Allam, B.W., Khalil, R.H., Basuini, M.F.E., Nour, A.A.M., Labib, E.M.H., Elkholy, I.S.E., Verdegem, M.C.J. and Abdel-Latif, H.M.R., Assessment of zootechnical parameters, intestinal digestive enzymes, haemato-immune responses, and hepatic antioxidant status of Pangasianodon hypophthalmus fingerlings reared under different stocking densities. *Aquaculture International*, 1-24 (2023).

55. Ayyat, M.S., Mahmoud, H.K., El-Hais, A.E.-A.M. and Abd El-Latif, K.M., The role of some feed additives in fish fed on diets contaminated with cadmium. *Environmental Science and Pollution Research*, **24**, 23636-23645 (2017).

56. Zhang, Q., Xie, Y., Qin, R., Huang, E., Zhang, Z., Zhou, J., Liu, D., Meng, L., Liu, Y. and Tong, T., Effects of cadmium on the growth, muscle composition, digestion, gene expression of antioxidant and lipid metabolism in juvenile tilapia (Oreochromis niloticus). *Frontiers in Marine Science*, *Sec. Aquatic Physiology*,**11**,1443484(2024). https://doi.org/10.3389/fmars.2024.1443484

57. Ferain, A., Delbecque, E., Neefs, I., Dailly, H., De Saeyer, N., Van Larebeke, M., Cornet, V., Larondelle, Y., Rees, J.F., Kestemont, P., De Schamphelaere, K.A.C. and Debier, C., Interplay between dietary lipids and cadmium exposure in rainbow trout liver: Influence on fatty acid metabolism, metal accumulation and stress response. *Aquatic toxicology (Amsterdam, Netherlands)*, **231** 105676 (2021).

58. Hani, Y.M.I., Turies, C., Palluel, O., Delahaut, L., Bado-Nilles, A., Geffard, A., Dedourge-Geffard, O., and Porcher, J.-M., Effects of a chronic exposure to different water temperatures and/or to an environmental cadmium concentration on the reproduction of the threespine stickleback (Gasterosteus aculeatus). *Ecotoxicology and Environmental Safety*, **174** 48-57 (2019).

59. Wu, H., Xuan, R., Li, Y., Zhang, X., Jing, W. and Wang, L., Biochemical, histological and ultrastructural alterations of the alimentary system in the freshwater crab Sinopotamon henanense subchronically exposed to cadmium. *Ecotoxicology (London, England)*, **23**(1), 65-75 (2014).

60. Attia, S.M., Varadharajan, K., Shanmugakonar, M., Das, S.C. and Al-Naemi, H.A., Cadmium: An Emerging Role in Adipose Tissue Dysfunction. *Exposure and Health*, **14**(1), 171-183 (2022).

61. Lucia, M., André, J.M., Gonzalez, P., Baudrimont, M., Bernadet, M.D., Gontier, K., Maury-Brachet, R., Guy, G. and Davail, S., Effect of dietary cadmium on lipid metabolism and storage of aquatic bird Cairina moschata. *Ecotoxicology (London, England)*, **19**(1), 163-170 (2010).

62. Abdel Rahman, A.N., Amer, S.A., Masoud, S.R., El-Saber, M.M., Osman, A., Younis, E.M., Abdelwarith, A.A., Davies, S.J., Khamis, T. and Ibrahim, R.E., Neem seed protein hydrolysate as a fishmeal substitute in Nile tilapia: Effects on antioxidant/immune pathway, growth, amino acid transporters-related gene expression, and Aeromonas veronii resistance. Aquaculture, 573 739593 (2023).

63. Abdel Rahman, A.N., Amer, S.A., Behairy, A., Younis, E.M., Abdelwarith, A.A., Osman, A., Moustafa, A.A., Davies, S.J. and Ibrahim, R.E., Using Azadirachta indica protein hydrolysate as a plant protein in Nile tilapia (Oreochromis niloticus) diet: Effects on the growth, economic efficiency, antioxidant-immune response and resistance to Streptococcus agalactiae. *Journal of Animal Physiology and Animal Nutrition*, **107**(6), 1502-1516 (2023).

64. da Silva, R.R., Enzymatic Synthesis of Protein Hydrolysates From Animal Proteins: Exploring Microbial Peptidases. *Frontiers in Microbiology*, **9**, 735 (2018). https://doi.org/10.3389/fmicb.2018.00735

65. Fazio, F., Fish hematology analysis as an important tool of aquaculture: a review. *Aquaculture*, **500**, 237-242 (2019).

66. Khadre, S., The effect of experimentally induced inflammation on the blood pattern and haemopoietic organs of the teleost, Clarias lazera. Bull. Inst. *Oceano. Fish. ARE*, **14**, 191-203 (1988).

67. Sharma, J. and Langer S., Effect of manganese on haematological parameters of fish, Garra gotyla gotyla. *J. Entomol. Zool. Stud.*, **2**(3), 77-81 (2014).

68. Witeska, M., Kondera, E. and Bojarski, B., Hematological and hematopoietic analysis in fish toxicology—a review. *Animals*, **13**(16), 2625 (2023).

69. Kyere, C., Twumasi, G., Seidu, H. and Quaye, T., Influence of neem (Azadirata indica) leaf meal on growth performance and blood profile of the pearl Guinea fowl. *Journal of Livestock Research for Rural Development*, **30** (30), 1- 5(2018).

70. Abarike, E., Dandi, S. and Ampofo-Yeboah A., A blend of Guava, Bitter, and Neem Leaf extracts improves haematology and resistance to co-infection of Streptococcus agalactiae and Aeromonas jandaie but not Liver health in Nile tilapia. *Fish and Shellfish Immunology Reports*, **3**, 100066 (2022).

71. Oniovosa, U.E., Aina, O.O., Alarape, S.A., Babalola, O.E. and Adeyemo, O.K., Effects of Neem Leaves Aqueous Extract on Organ Histology, Haematological Parameters and Biochemical Indices in Catfish. *Alexandria Journal for Veterinary Sciences*, **54**(1), (2017).

72. Munugalavadla, V., Borneo, J., Ingram, D.A. and Kapur R., p85α subunit of class IA PI-3 kinase is crucial for macrophage growth and migration. *Blood*, **106**(1), 103-109 (2005).

73. Gadan, K., Marjara, I.S., Sundh, H., Sundell, K. and Evensen, Ø., Slow release cortisol implants result in impaired innate immune responses and higher infection prevalence following experimental challenge with infectious pancreatic necrosis virus in Atlantic salmon (Salmo salar) parr. *Fish Shellfish Immunol.*, **32**(5), 637-644 (2012).

74. Bautista-Covarrubias, J.C., Valdez-Soto, I.E., Aguilar-Juárez, M., Arreola-Hernández, J.O., Soto-Jiménez, M.F., Soto-Rodríguez, S.A., López-Sánchez, J.A., Osuna-Martínez, C.C. and Frías-Espericueta M.G., Cadmium and copper mixture effects on immunological response and susceptibility to Vibrio

harveyi in white shrimp Litopenaeus vannamei. *Fish & Shellfish Immunology*, **129**, 145-151 (2022).

75. Xia, H., Ding, C., Zhang, Y., Liu, L., He, H., Zhou, W., Liu, H., Chen, F., Bu, J. and Yu, J., Adverse effects of cadmium exposure on the immune and antioxidant system functions of dongtingking crucian carp (Carassius auratus indigentiaus). *Aquaculture Reports*, **39**, 102516 (2024).

76. Jabeen, G., Ishaq, S., Arshad, M., Fatima, S., Kanwal, Z., and Ali, F., Investigation on Immune-Related Protein (Heat Shock Proteins and Metallothionein) Gene Expression Changes and Liver Histopathology in Cadmium-Stressed Fish. *Biomed. Research International*, **2022**, 2075791 (2022).

77. Asghar, H.A., Abbas, S.Q., Arshad, M.K., Jabin, A., Usman, B., Aslam, M. and Asghar, A., Therapeutic potential of Azadirachta indica (Neem)-a comprehensive review. *Sch. Int. J. Tradit. Complement. Med.*, **5**, 47-64 (2022).

78. Graßmann, J., Terpenoids as plant antioxidants. *Vitamins & Hormones*, **72**, 505-535 (2005).

79. Abdel-Razek, N., El-Sabbagh, N., Khalil, R.H. and Abdel-Tawwab, M., Prophylactic effects of dietary caper (Capparis spinosa) extracts on the control of Streptococcus agalactiae infection, growth, immune-antioxidant, and inflammation cytokine responses of Nile tilapia fingerlings. *Fish & Shellfish Immunology*, **142**, 109126 (2023).

80. Heydarnejad, M.S., Khosravian-Hemamai, M. and Nematollahi, A., Effects of cadmium at sub-lethal concentration on growth and biochemical parameters in rainbow trout (Oncorhynchus mykiss). *Irish Veterinary Journal*, **66**, 1-7 (2013).

81. Kori-Siakpere, O., Ake, J. and Avworo, U., Sublethal effects of cadmium on some selected haematological parameters of Heteroclarias (a hybrid of Heterobranchus bidorsalis and Clarias gariepinus). *International Journal of Zoological Research*, **2**(1), 77-83 (2006).

82. Kumar, K., A review of heavy metals toxicological studies on mangur (Clarias batrachus) fish. *International Journal of Engineering and Management Research (IJEMR)*, **6**(5), 577-583 (2016).

83. Al-Asgah, N.A., Abdel-Warith, A.-W.A., Younis, E.-S.M. and Allam, H.Y., Haematological and biochemical parameters and tissue accumulations of cadmium in Oreochromis niloticus exposed to various concentrations of cadmium chloride. *Saudi Journal Of Biological Sciences*, **22**(5), 543-550 (2015).

84. Fontana, L., Moreira, E., Torres, M.I., Fernández, I., Ríos, A., de Medina, F.S., and Gil, A., Dietary nucleotides correct plasma and liver microsomal fatty acid alterations in rats with liver cirrhosis induced by oral intake of thioacetamide. *Journal Of Hepatology*, **28** (4), 662-669 (1998).

85. Gad, N.S., Impact of environmental pollution in the southern region of lake Manzalah Egypt on some biochemical parameters of Tilapia zillii. Journal-*Egyptian German Society of Zoology*, **48**(A), 279 (2005).

86. Elarabany, N., Bahnsawy, M. and Alfakheri, M., Effects of cadmium on some haematological and biochemical parameters of African catfish, Clarias graiepinus. *African Journal of Biological Sciences*, **15**(1), 43-52 (2019).

87. El-Boshy, M.E.-S., Gadalla, H.A. and El-Hamied, F., Immunological, hematological and biochemical changes induced by short term exposure to cadmium in catfish (*Clarias gariepinus*). *J. Coast. Life Med*., **2**(3), 175-180 (2014).

88. Fırat, Ö. and Kargın, F., Individual and combined effects of heavy metals on serum biochemistry of Nile tilapia Oreochromis niloticus. *Archives of Environmental Contamination and Toxicology*, **58** 151-157 (2010).

89. Fabisiak, J.P., Sedlov, A. and Kagan, V.E., Quantification of oxidative/nitrosative modification of CYS34 in human serum albumin using a fluorescence-based SDS-PAGE assay. *Antioxidants and Redox Signaling*, **4**(5), 855-865 (2002).

90. Bernet, D., Schmidt, H., Wahli, T. and Burkhardt-Holm, P., Effluent from a sewage treatment works causes changes in serum chemistry of brown trout (Salmo trutta L.). *Ecotoxicology And Environmental Safety*, **48**(2), 140-147 (2001).

91. Yousefi, S., Shokri, M.M., Noveirian, H.A. and Hoseinifar, S.H., Effects of dietary yeast cell wall on biochemical indices, serum and skin mucus immune responses, oxidative status and resistance against Aeromonas hydrophila in juvenile Persian sturgeon (Acipenser persicus). *Fish & Shellfish Immunology*, **106**, 464-472 (2020).

92. Zardooz, H., Asl, S.Z., Naseri, M.K.G, and Hedayati, M., Effect of chronic restraint stress on carbohydrate metabolism in rat. *Physiology & Behavior*, **89**(3), 373-378 (2006).

93. Kazlauskienė, N. and Vosylienė, M.Z., Peculiarities of the physiological responses of rainbow trout (Oncorhynchus mykiss) to copper. *Acta Zoologica Lituanica*, **9**(2), 56-70 (1999).

94. Gaber, H.S., El-Kasheif, M.A., Ibrahim, S.A. and Authman M., Effect of water pollution in El-Rahawy drainage canal on hematology and organs of freshwater fish. *World Applied Sciences Journal*, **21**(3), 329-341 (2013).

95. Adamu, K.M., Sublethal effects of tobacco (Nicotiana tobaccum) leaf dust on enzymatic activities of Heteroclarias (a hybrid of Heterobranchus bidorsalis and Clarias gariepinus). *Jordan Journal of Biological Sciences*, **2**(4), 151-158 (2009).

96. Yarmohammadi, F., Mehri, S., Najafi, N., Amoli, S.S. and Hosseinzadeh, H., The protective effect of Azadirachta indica (neem) against metabolic syndrome: A review. *Iranian Journal of Basic Medical Sciences*, **24**(3), 280 (2021).

97. Gomase, P.V., Rangari, V.D. and Verma, P., Phytochemical evaluation and hepatoprotective activity of fresh juice of young stem (tender) bark of Azadirachta indica A. Juss. *International Journal of Pharmacy and Pharmaceutical Sciences*, **3**(2), 55-59 (2011).

98. Ghiasi, F., Mirzargar, S.S., and Ashrafihellan, J., Histological effects of cadmium on hepatopancreas and gill in Cyprinus carpio. *Iranian Journal of Toxicology*, **11**(5), 1-4 (2017).

99. El-Sokkary, G.H., Nafady, A.A. and Shabash, E.H., Melatonin administration ameliorates cadmium-induced oxidative stress and morphological changes in the liver of rat. *Ecotoxicology And Environmental Safety*, **73**(3), 456-463 (2010).

100. Arroyo, V., Flores, K., Ortiz, L., Gómez-Quiroz, L. and Gutiérrez-Ruiz, M., Liver and cadmium toxicity. *J. Drug Metab. Toxicol. S.*, **5**(001), 85, 1-7 (2012).

101. Basha, P.S. and Rani, A.U., Cadmium-induced antioxidant defense mechanism in freshwater teleost Oreochromis mossambicus (Tilapia). *Ecotoxicology and Environmental Safety*, **56**(2), 218-221 (2003).

102. Drąg-Kozak, E., Pawlica-Gosiewska, D., Gawlik, K., Socha, M., Gosiewski, G., Łuszczek-Trojnar, E., Solnica, B. and Popek, W., Cadmium-induced oxidative stress in Prussian carp (Carassius gibelio Bloch) hepatopancreas: ameliorating effect of melatonin. *Environmental Science and Pollution Research*, **26** 12264-12279 (2019).

103. Liu, X., Deng, Q., Yang, H., Wang, J. and Wang, M., Oxidative stress of Cadmium and Lead at environmentally relevant concentrations on hepatopancreas of macrobrachium nipponensis and their mixture interactivity: Implications for water quality criteria amendment. *International Journal of Environmental Research and Public Health*, **20**(1), 360 (2022).

104. Zhang, Q., Xie, Y., Qin, R., Huang, E., Zhang, Z., Zhou, J., Liu, D., Meng, L., Liu, Y. and Tong, T., Effects of cadmium on the growth, muscle composition, digestion, gene expression of antioxidant and lipid metabolism in juvenile tilapia (Oreochromis niloticus). *Frontiers in Marine Science*, **11**, 1443484 (2024).

105. Messaoudi, I., Barhoumi, S., Saïd, K. and Kerken A., Study on the sensitivity to cadmium of marine fish Salaria basilisca (Pisces: Blennidae). *Journal of Environmental Sciences*, **21**(11), 1620-1624 (2009).

106. Boyes, W.K., Bingham, E., Cohrssen, B. and Powell, C.H., Neurotoxicology and behavior. Patty's Toxicology, 5th ed., John Wiley & Sons Inc., New York, NY, 35-74 (2012).

107. Lee, D.-C., Choi, Y.J. and Kim, J.-H., Toxic effects of waterborne cadmium exposure on hematological parameters, oxidative stress, neurotoxicity, and heat shock protein 70 in juvenile olive flounder, Paralichthys olivaceus. *Fish & Shellfish Immunology*, **122**, 476-483 (2022).

108. Ibrahim, R.E., Elshopakey, G.E., Abdelwarith, A.A., Younis, E.M., Ismail, S.H., Ahmed, A.I., El-Saber, M.M., Abdelhamid, A.E., Davies, S.J. and El-Murr, A., Chitosan neem nanocapsule enhances immunity and disease resistance in nile tilapia (Oreochromis niloticus). *Heliyon*, **9**(9), e19354 (2023).

109. Ibrahim, D., Abd El-Hamid, M.I., Al-Zaban, M.I., ElHady, M., El-Azzouny, M.M., ElFeky, T.M., Al Sadik, G.M., Samy, O.M., Hamed, T.A. and Albalwe, F.M., Impacts of fortifying Nile tilapia (Oreochromis niloticus) diet with different strains of microalgae on its performance, fillet quality and disease resistance to Aeromonas hydrophila considering the interplay between antioxidant and inflammatory response. *Antioxidants*, **11**(11), 2181 (2022).

110. Abu-Elala, N.M., Khattab, M.S., AbuBakr, H.O., Helmy, S., Hesham, A., Younis, N.A., Dawood, M.A., and El Basuini, M.F., Neem leaf powder (Azadirachta indica) mitigates oxidative stress and pathological alterations triggered by lead toxicity in Nile tilapia (Oreochromis niloticus). *Scientific Reports*, **13**(1), 9170 (2023).

111. Marijić, V.F. and Raspor, B., Age-and tissue-dependent metallothionein and cytosolic metal distribution in a native Mediterranean fish, Mullus barbatus, from the Eastern Adriatic Sea. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **143**(4), 382-387 (2006).

112. Erdem, C. and Kargin, F., A comparative study on the accumulation of copper in liver, spleen, stomach, intestine, gill and muscle tissues of Cyprinus carpio and Tilapia nilotica. *Biyokim. Derg.*, **1**, 13-27 (1992).

113. Sharma, A. and Bhattacharyya, K.G., Azadirachta indica (Neem) leaf powder as a biosorbent for removal of Cd(II) from aqueous medium. *Journal of Hazardous Materials*, **125**(1), 102-112 (2005).

114. Awad, E., Cordero, H., Esteban, M., Soliman, W.S., Abbas, W.T., and Ibrahim, T.B., Immune and Antioxidant-Related Genes in the Nile Tilapia (Oreochromisniloticus) Collected From Different Locations. *Egyptian Journal of Aquatic Biology & Fisheries*, **27**(6), 3-17(2023).

115. Standen, B., Peggs, D., Rawling, M., Foey, A., Davies, S., Santos, G. and Merrifield, D., Dietary administration of a commercial mixed-species probiotic improves growth performance and modulates the intestinal immunity of tilapia, Oreochromis niloticus. *Fish & Shellfish Immunology*, **49**, 427-435 (2016).

116. Qiang, J., He, J., Yang, H., Xu, P., Habte-Tsion, H.M., Ma, X., and Zhu, Z., The changes in cortisol and expression of immune genes of GIFT tilapia Oreochromis niloticus (L.) at different rearing densities under Streptococcus iniae infection. *Aquaculture International*, **24**, 1365-1378 (2016).

117. Pang, J.-c., Gao, F.-y., Lu, M.-x., Ye, X., Zhu, H.-p. and Ke, X.-l., Major histocompatibility complex class IIA and IIB genes of Nile tilapia Oreochromis niloticus: genomic structure, molecular polymorphism and expression patterns. *Fish & Shellfish Immunology*, **34**(2), 486-496 (2013).

التأثيرات المُحسّنة لمسحوق أوراق النيم على سُمّية الكادميوم في أسماك البلطي النيلي: أداء النمو، والكيمياء الحيوية الدموية، والأكسدة، والتحليلات المرضية

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الملخص

سعى بحثنا إلى توضيح تأثير مسحوق أوراق النيم (NP) كمكمل غذائي في الحد من الآثار الضارة لسمية كلوريد الكادميوم في أسماك البلطي النيلي. قُسِّمت 360 سمكة مُتأقلمة عشوائيًا إلى 4 تجارب، بواقع 3 تكرارات لكل مجموعة، بما في ذلك المجموعة الضابطة، والمجموعة المُسمَّمة بكلوريد الكادميوم (2 ملغم/لتر في الماء)، ومجموعتان غُذيتا بمسحوق أوراق النيم بجرعات 2 و4 غ/كغ من العلف مع التعرض لـ 2 ملغم/لتر من كلوريد الكادميوم لمدة 60 يومًا. تم قياس إنزيمات الجهاز الهضمي، والبروتين الكلي، والألبومين، ومؤشرات وظائف الكبد (AST, ALT, LDH) والكوليسترول الكلي، والدهون الثلاثية، بالإضافة إلى المؤشرات الحيوية لأكسدة/مضادات أكسدة الكبد) سوبر أكسيد ديسميوتاز، كاتالاز، غلوتاثيون بيروكسيديز، غلوتاثيون S ترانسفيراز (باستخدام جهاز مطياف ضوئي. تم الكشف عن التعبير الجيني لـ PCNA في الأمعاء باستخدام الكيمياء المناعية. أظهرت بياناتنا أن أسماك البلطي المعرضة لسمية كلوريد الكادميوم في الماء أظهرت انخفاضًا ملحوظًا في أداء النمو، واستخدام العلف، ومعدلات بقاء الأسماك، وإنزيمات الهضم المعوية (البروتياز، والليباز، والأميليز). علاوة على ذلك، أظهرت الأسماك المعرضة لسمية كلوريد الكادميوم انخفاضًا ملحوظًا في المعايير الدموية، والبروتين الكلي، والغلوبيولين، وبعض مؤشرات مضادات الأكسدة، بما في ذلك الكاتالاز، وغلوتاثيون إس-ترانسفيراز، والغلوتاثيون الكلي والمختزل، بالإضافة إلى سوبر أكسيد ديموتاز. بالإضافة إلى ذلك، أدت سمية كلوريد الكادميوم إلى زيادة ملحوظة في مستويات الألبومين، والجلوكوز، وإنزيمات الكبد، والكوليسترول الكلي، والدهون الثلاثية، وغلوتاثيون بيروكسيديز، وبيروكسيد الدهون .(LPO) وزادت بقايا كلوريد الكادميوم في الكبد والخياشيم والعضلات بشكل ملحوظ، وخاصة في أنسجة الكبد في المجموعة المعرضة لسمية كلوريد الكادميوم. أدت المعالجة باستخدام النيم بتركيزين (2 و4 غ/كغ) من النظام الغذائي إلى الحد من هذه التأثيرات الضارة من خلال زيادة تنظيم معايير النمو، والمعايير الدموية، ونشاط الإنزيمات المعوية والكبدية ومضادات الأكسدة، مع انخفاض تنظيم معايير LPO ومستوى الدهون، مع تحسن في البنية النسيجية المعوية.

الكلمات الدالة: مسحوق النيم، سمك البلطي، الكادميوم، أداء النمو.