

Elucidating the Role of Prickly Pear Fruits (*Opuntia littoralis*) in Mitigation of Cadmium Toxicity in *Oreochromis niloticus*: Impacts on Haemato-Biochemical and Immunological Responses

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Abstract

The purpose of the study was to ascertain the preventative value of dietary supplements with fruits of the prickly pear on the haemato-biochemical and immunological responses of *O. niloticus* exposed to subacute waterborne cadmium toxicity. Four diets supplemented with different proportions of fruits of 0% (control, OLFE-0% group), 0.5% (OLFE-0.5% group), 1% (OLFE-1% group), and 2% (OLFE-2% group) were administered to tilapia fingerlings for sixty days (pre-cadmium). After the feeding session, the tilapia treatments were subjected to waterborne cadmium toxicity for four days (post-cadmium). Blood samples were captured pre- and post-cadmium exposure to assess the haemato-biochemical and immunological alternations. According to the current findings, nutritional meals fortified with OLFE can enhance tilapia fish's growth performance and digestive enzymes. Nile tilapia fed on OLFE-fortified diets showed a significant reduction in cortisol, alanine transaminase (ALAT), aspartate transaminase (ASAT), glucose, protein, and malondialdehyde (MDA) compared to the control with a basal diet without OLFE ($P < 0.05$). Additionally, it significantly improves the activities of complement C3, lysozyme, catalase (CAT), total immunoglobulin, superoxide dismutase (SOD), and glutathione peroxidase (GPX). Significant increases in cortisol, leukocytes, glucose, CAT, ALAT, GPX, ASAT, and SOD were observed following exposure to waterborne cadmium. At the same time, there were decreases in erythrocytes, blood indices, hemoglobin, complement C3, packed cell volume, lysozyme, total immunoglobulin, and malondialdehyde (MDA) values. In brief, these findings suggest that supplementing prickly pear fruits to tilapia diets, especially at a level of 2%, can enhance immunological and antioxidant properties and effectively mitigate the harmful effects of Cd exposure in food safety and aquaculture.

Introduction

Aquaculture contributes significantly to the global food supply and is one of the agricultural industries with the quickest growth due to its high demand (FAO 2022 & Bjørndal and Dey 2023). Due to its rapid development, *Oreochromis niloticus*, also known as Nile tilapia, is one of the most widely cultivated fish in Egypt and other parts of the world, with exceptional adaptability to a wide range of environments and considerable commercial income (Abdo et al. 2024). Fish reside at the top of aquatic environments and have a higher trophic rank in the food chain (Kwaansa-Ansah et al. 2019); they play a major role in delivering pollutants to humans (Verbeke et al. 2005).

Toxic metals accumulate in fish at minor levels via bioaccumulation and at higher levels via the biomagnification process; however, people may consume metals via food consumption, which may cause adverse health consequences in the short term (Abbas et al. 2023 and Schenone et al. 2014). In aquatic ecosystems, heavy metals are one of the most common pollutants absorbed by fish and transferred to upper trophic levels through food or the environment (Miri et al. 2017). Toxic metals, like Arsenic, Cadmium, Chromium (VI), and Lead, have no biological significance in any manner. In contrast, essential metals, like Chromium (III), Copper, Zinc, Manganese, and Nickel, are required at trace levels for a variety of physiological and enzymatic activities (Miedico et al. 2015). A great deal more emphasis needs to be placed on metals since they are not biodegradable, persist in the aquatic environment over an

extended period and consequently accumulate at progressively higher levels in the food chain via biomagnification (Bettini et al. 2006). Of these metals, cadmium is a hazardous element that has an impact on cultivated fish's physiological state. Considering cadmium may readily accumulate in the food chain and aquaculture tissues, it has become an important environmental issue on a worldwide scale. It may potentially have adverse impacts on human health (Hui et al. 2022). Fish experience high bioaccumulation of Cd through dietary consumption, exchange of ions via transmembrane and gills, and tissue adsorption from the aquatic environment (Ahmed et al. 2014). The prolonged accumulation of heavy metals in the tissues of aquatic organisms may alter their immune systems, putting them at risk for oxidative injury (Raeeszadeh et al. 2023). Among other consequences, it may result in anemia, acute pathological impairment, and abnormalities related to biochemical, physiological, and oxidative stress (Otludil et al. 2017; Abdel-Tawwab and Wafeek 2010; Al-Asgah et al. 2015). Physiological responses to environmental challenges in aquatic species refer to changes to their growth, well-being, behavior, metabolic range of activity, overall sickness resistance, and, eventually, their survival ability (Zeitoun et al. 2016). Mitigation of the adverse impacts of cadmium toxicity on aquatic organisms has a significant influence on sustainable aquaculture. Therefore, the demand for affordable, long-lasting, and effective methods to lessen the harmful impacts of toxic metals on fish health is critical. Finding novel compounds or widely available dietary supplements to improve fish development and physiological markers is therefore of increasing interest.

Supplements for a fish diet are utilized to improve fish health and tolerance to disease and toxins. Numerous investigations have demonstrated that supplementing fish diets with medicinal herbs can help mitigate the negative effects of toxicants, such as cadmium toxicity, in different fish species (Sarwar et al. 2010; Hoseini et al. 2019; Taheri Mirghaied et al. 2019; Zhang et al. 2019; Yilmaz 2020; Abdelzaher et al. 2022b; Abdel-Tawwab et al. 2020 and Gehad et al. 2023). Medicinal herbs can function as immunostimulants by providing early activation to fish's non-specific defense mechanisms and enhancing the immune response. Phytochemicals are beneficial components in plants, and their extracts include carotenoids, phenolics, flavonoids, and many more (Abdel-Latif et al. 2023). These substances' growth-promoting, antioxidant, antimicrobial, immunostimulant, and health-promoting properties have made them extremely important (Ahmadifar et al. 2021 & Naiel et al. 2023).

Prickly pears are widely utilized in the food, pharmaceutical, and medical industries. Among the Cactaceae family, *Opuntia littoralis* is an edible plant with therapeutic uses that are widely used worldwide and employed in diets for poultry and animals (Mahrose 2021). Numerous active phytochemical ingredients, including terpenes, flavonoids, tannins, carbohydrates, coumarins, and glycosides, are present in the prickly pear fruits (Stintzing and Carle 2005 & Galal et al. 2017). According to Ahmadifar et al. (2021) and Saheli et al. (2021), these substances may be extremely important in protecting aquatic life from the damaging effects of reactive oxygen species (ROS) or nitrogen. They also exhibit immunological, antibacterial, and antioxidant action. Herbal dietary supplements have been shown in prior research on cadmium toxicity to reduce the effects of waterborne cadmium on immunosuppression, oxidative stress, and stress reactions (Zhou et al. 2017; Elgendy et al. 2023 and Abdel-Tawwab et al. 2024). Still, no research was conducted to elucidate the possible use of dietary

supplements that include prickly pear fruits in tilapia diets following exposure to cadmium toxicity. Thus, the purpose of the study was to assess the growth-promoting impacts of supplemented diets with fruits of *Opuntia littoralis* and its potential benefits to mitigate the adverse effects of cadmium exposure. The study will involve a sixty-day feeding with Nile tilapia fed on a diet supplemented with fruits of *O. littoralis* (pre-cadmium), followed by exposure to cadmium toxicity for four days (post-cadmium).

Materials and methods

Sampling collected.

Nile tilapia was collected from Kafr El-Sheikh fishponds in Egypt. Samples were carefully brought into the laboratory to be examined. Upon arriving at the lab, they were placed in glass aquariums for fourteen days to allow the fish to acclimate. During the visual assessment (Radwan et al. 2023), no obvious diseases or injuries were found on the fish specimens, which appeared to be in good condition. Similarly, fresh fruits of prickly pears (*O. littoralis*) were gathered in the summer of 2023 from the Wady-Mageid location in Egypt's Marsa-Matrouh province.

Preparation of prickly pear extract

The fresh fruits of the prickly pear, *O. littoralis*, were washed and left to dry in the shade for a few days. However, according to Abd El-Moaty (2020), the fruits were extracted using Soxhlet equipment and solvents containing 70% ethanol. The extracted fruits were kept in a zip-lock bag at 4°C until use. The four levels of extracted fruits of prickly pears (OLFE-0): 0% (control, OLFE-0 group), 0.5% (OLFE-1 group), 1% (OLFE-2 group), and 2% (OLFE-3 group) were used to prepare the formulated diets in the study (Table 1). Each prepared diet was compressed using a mincer after the feed components of four extracted fruit groups were mixed with oil and water to form a solid paste. The pellets from the four diet groups were air-dried and placed into plastic containers at 4°C until needed (Hoseinifar et al. 2020).

Table 1
Formulation and body composition of OLFE-fortified diets (% , on a dry basis).

Ingredients	OLFE-fortified diets			
	OLFE-0% (Control)	OLFE-0.5%	OLFE-1%	OLFE-2%
Fish meal (72.0% CP)	9.98	9.98	9.98	9.98
Soybean meal (48% CP)	42.02	42.02	42.02	42.02
Yellow corn	19.50	19.50	19.50	19.50
Wheat flour	9.06	8.56	8.06	7.06
Wheat bran	15.50	15.50	15.50	15.50
Vegetable oil	1.30	1.30	1.30	1.30
Cod liver oil	1.25	1.25	1.25	1.25
Dicalcium phosphate	0.90	0.90	0.90	0.90
1Vitamine and mineral mixture	0.18	0.18	0.18	0.18
Vitamin C	0.31	0.31	0.31	0.31
OLFE	0	0.5	1	2
	100	100	100	100
Proximate chemical analysis (%)				
Moisture (%)	10.12	10.13	10.09	10.13
Dry matter (%)	89.88	89.87	89.91	89.87
Crude protein (%)	31.10	31.14	31.12	31.10
Crude lipid (%)	8.39	8.42	8.44	8.45
Fiber (%)	6.29	6.19	6.22	6.23
Ash (%)	10.68	10.70	10.71	10.70
Nitrogen-free extract (%)	43.53	43.55	43.51	43.52
Gross energy	1997.99	2000.47	2000.26	2000.19
* Mixtures of vitamins and minerals in each 1 kg, including 700 000 IU of Vit. A, 700 000 IU of Vit. D3, 4000 mg of Vit. E, 670 mg of Vit. K3, 350 mg of Vit. B1, 1700 mg of Vit. B2, 500 mg of Vit. B6, 3.5 mg of Vit. B12, 19 mg of Biotin, 4000 mg of Pantothenic Acid, 10000 mg of Nicotinic Acid, 450 mg of Folic Acid, 21000 mg of Mn, 19000 mg of Zn, 11000 mg of Fe, 3500 mg of Cu, 350 mg of Iodine, 350 mg of Selenium, 35 mg of Co, add to 1 kg of CaCo3 as carrier.				

GC-MS identification of prickly pear extract

The chemical structure of the concentrated fruit extract was analyzed and determined using gas chromatography and mass spectrometry (GC-MS). Identification of the compound was accomplished by comparing the mass spectral library of the GC-MS information system (Sigma-Aldrich) with chromatographic retention properties. Compound quantification was accomplished using the total ion peak area and calibration curves of the external standards (Agricultural Research Center, Dokki, Giza). The composition of bioactive compounds is summarized in Table 2.

Table 2
The GC-MS of *Opuntia littoralis* fruit extract (OL-FE) showed the identified components.

No	Compound Identified	M.wt	Formula	RT (min)	Area (%)
1	Malic acid	134.08	C ₄ H ₆ O ₅	6.14	55.36
2	Quinic acid	192.17	C ₇ H ₁₂ O ₆	6.88	53.21
3	Gallic acid	170.12	C ₇ H ₆ O ₅	7.91	51.74
4	Protocatechuic acid	154.12	C ₇ H ₆ O ₄	8.14	48.59
5	Chlorogenic acid	354.31	C ₁₆ H ₁₈ O ₉	8.96	47.19
6	p-Coumaric acid	164.04	C ₉ H ₈ O ₃	9.22	42.11
7	Ursolic acid	456.7	C ₃₀ H ₄₈ O ₃	10.40	38.93
8	Caffeic acid	180.16	C ₉ H ₈ O ₄	14.95	33.32
9	Gomphrenin-I	550.5	C ₂₄ H ₂₆ N ₂ O ₁₃	15.46	31.02
10	Musca-aurin-I	351.27	C ₁₄ H ₁₃ N ₃ O ₈	15.88	28.82
11	Vulgaxanthin-I	339.30	C ₁₄ H ₁₇ N ₃ O ₇	16.77	26.44
12	Betalamic acid	211.17	C ₉ H ₉ NO ₅	17.72	23.72
13	Beta-Carotene	536.9	C ₄₀ H ₅₆	18.52	19.22
14	Zeaxanthin	215.5	C ₄₀ H ₅₆ O ₂	19.33	18.47
15	α-Cryptoxanthin	552.87	C ₄₀ H ₅₆ O	21.23	17.66
16	ascorbic acid	176.12	C ₆ H ₈ O ₆	22.39	16.33
17	α-Tocopherol	430.71	C ₂₉ H ₅₀ O ₂	24.71	12.28
18	Beta-Sitosterol	414.7	C ₂₉ H ₅₀ O	26.79	10.27
19	strictosidine aglycone	368.43	C ₂₁ H ₂₄ N ₂ O ₄	28.42	8.91
20	campesterol 3-β-D-glucoside	562.83	C ₃₄ H ₅₈ O ₆	29.37	7.43

Feeding experiment

The acclimatized, homogenous, and healthy tilapia fish in four groups (OLFE-0%, OLFE-0.5%, OLFE-1%, and OLFE-2%) were selected at random and assigned to twelve glass aquariums with a 100-liter water volume, each with three replicates (fifteen fish per aquarium). The fish of the diet groups were fed at an

average of 3% of their body weight for sixty days between 9:00 a.m. and 4:00 p.m. During the feeding trial, biometry was carried out every 15 days (three times) to recalculate feed intake and visually assess the health condition of the fish. Each aquarium was continuously aerated, siphoned, and refilled daily with 70% dechlorinated water. The water quality of every aquarium was routinely monitored during the trial: $26.6 \pm 0.57^{\circ}\text{C}$ for water temperature, 7.63 ± 0.38 for pH, 6.6 ± 0.74 mg/L for dissolved oxygen, and 0.19 ± 0.02 mg/L for total ammonia nitrogen.

Toxicity and challenge experiment of cadmium

Following the acclimatization duration, pilot studies were carried out to identify the median lethal concentration of cadmium nitrate at 96 hours. Cadmium nitrate ($\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) stock solution of 1,000 ppm was prepared, and seven 100 L glass aquaria were filled with different levels of cadmium (0 (control without Cd), 10, 12.5, 15, 17.5, 20, 22.5, and 25 ppm of Cd stock solution). Each aquarium contained 10 fish and was subjected to levels of cadmium for ninety-six hours. There were two replications of the treatment and control tests. The American Public Health Association methodology (APHA 2005) was used to establish the median lethal level at 96 hours (96 h LC_{50}), which came out to be 18.6 ppm. Fish OLFE diets were fasted twenty-four hours before the cadmium challenge. 50% of the established 96-hour LC_{50} , or 9.3 ppm of cadmium, was subjected to each of the four OLFE groups for 4 days. Fish were sampled for investigation after being exposed to cadmium (post-cadmium).

Blood sample collection

To assess the blood variables, the fish were fasted for twenty-four hours and anesthetized with clove oil (fifty $\mu\text{L/L}$) after the feeding trial (pre-cadmium) and the cadmium toxicity (post-cadmium). The caudal peduncle of three fish in each aquarium was examined with a syringe loaded with EDTA as an anticoagulant, which was employed in the hematological analysis. On the other hand, the immunological, biochemical, and antioxidative samples were obtained with a syringe (without anticoagulants), centrifuged at room temperature for 15 minutes at 3000 rpm to extract serum, and then frozen at -20°C to await further investigation. Investigations were conducted on immunological and haemato-biochemical alternations in pre-cadmium and post-cadmium samples.

Proximate composition analysis

The proximate chemical composition Table 1: Ash, crude protein, crude fiber, total lipids, and dry matter in the designed diets were examined (Thiex et al. 2012). The following method was used to compute the nitrogen-free extract (NFE): $\text{NFE} (\%) = 100 - (\text{Ash} (\%) + \text{crude protein} (\%) + \text{fiber} (\%) + \text{crude lipid} (\%))$. Based on the estimates of 23.6 for protein, 17.2 for carbohydrate, and 39.5 KJ/g for lipid, the gross energy of the diet was calculated.

Growth Performance and feed efficiency measurements

The growth performance and feed efficiency measurements were conducted for the tilapia groups, and the tilapia survival rate = $((\text{final tilapia number} - \text{initial tilapia number}) / (\text{initial tilapia number} \times 100))$; tilapia weight gain (g) = $[\text{final tilapia weight} - \text{initial tilapia weight}]$; feed conversion ratio = (tilapia feed

intake (g) / tilapia weight gain (g)); and specific growth rate (%day⁻¹) = $\frac{[(\text{LN final tilapia weight} - \text{LN initial tilapia weight}) / (\text{number of days})] \times 100}{}$ were calculated.

Lipase, protease, and amylase measurements

The digestive enzymes lipase, protease, and amylase were measured in tilapia intestine samples. Fish (three intestines) specimens were obtained from each aquarium, cut, homogenized, and centrifuged at 4°C, and then the extracted supernatant was extracted as described by Najdegerami et al. (2016). The activities of intestinal amylase and protease were evaluated using starch (0.3%) and casein (1%) as substrates, respectively (Langlois et al. 1987 & Iversen and Jørgensen, 1995). However, the lipase activity was reported using the p-nitrophenyl myristate hydrolysis method (Iijima et al. 1998).

Haemato-biochemical, immunological, and antioxidative assays

Hematological measurements (packed cell volume, hemoglobin, erythrocytes, leucocytes, and blood cell indices) were detected in blood EDTA tilapia, as described by Brown (1993), Van Kampen and Zijlstra (1983), and Dacie and Lewis (1991). However, blood glucose (Trinder 1969) and cortisol levels (Vecsei 1979) were reported using Bio-Merieux France kits. Tilapia biochemical parameters (total protein and activities of ALT and AST) were measured in the blood serum of tilapia, as reported by Henry (1964) and Reitman & Frankel (1957). Creatinine and lipid levels in tilapia serum were described by Pincus (1996). Tilapia immunological measurements (complement C3, total immunoglobulin, and lysozyme activities) in the blood serum of tilapia, as reported by Siwicki & Anderson (1993) and Tang et al. (2008). Tilapia antioxidative measurements in serum (superoxide dismutase, glutathione peroxidase, catalase, and malonaldehyde activities) were determined with diagnostic kits.

Statistical analysis

Levene's test was used to check the acquired data (mean ± SE) for homogeneity and normality. Using the SPSS software (Version, 22), two-way ANOVA measurements were performed between the data of the OLFE groups in pre-cadmium, post-cadmium, and their interactions, alongside "Excel 365" for creating figures. A Tukey test was employed if significant differences ($P < 0.05$) were observed between variables. The pre-cadmium and post-cadmium sample differences were identified using the T-test.

Results and Discussion

Growth performance of tilapia

Table 3 shows the impact of OLFE-fortified diets on the following metrics: specific growth rate, feed intake, initial tilapia weight, feed conversion ratio, final tilapia weight, tilapia weight gain, and survival rate. When the amount of OLFE in the diet increased, the growth performance of tilapia showed improvements in all variables, with a significant difference between OLFE levels ($P < 0.05$). The diet

designated as OLFE-3 had the maximum growth performance, trailed by the OLFE-2, OLFE-1, and OLFE-0 groups (control without OLFE).

Table 3

Growth performance and digestive enzymes of tilapia fish fed OLFE-fortified diets (OLFE) for sixty days.

Growth performance	OLFE levels (%)				p-value
	OLFE-0%	OLFE-0.5%	OLFE-1%	OLFE-2%	
Initial body weight (g (	21.30 ± 1.35	21.54 ± 1.85	21.25 ± 1.11	22.10 ± 1.66	0.23
Final weight (g (	53.66 ± 2.97 ^c	67.89 ± 3.37 ^b	69.24 ± 1.85 ^b	78.65 ± 2.27 ^a	0.04
Weight gain (g (	32.36 ± 3.13 ^c	46.35 ± 3.74 ^b	47.99 ± 1.94 ^b	56.55 ± 3.10 ^a	0.03
Specific growth rate (%/day)	1.54 ± 0.11 ^c	1.91 ± 0.13 ^b	1.97 ± 0.07 ^a	2.12 ± 0.11 ^a	0.02
Feed intake (g)	48.65 ± 3.32 ^c	59.35 ± 3.09 ^b	53.25 ± 3.23 ^b	68.90 ± 4.23 ^a	0.04
Feed conversion ratio	1.50 ± 0.21 ^a	1.28 ± 0.25 ^b	1.32 ± 0.10 ^b	1.22 ± 0.15 ^c	0.01
Survival rate (%)	100	100	100	100	
Digestive enzymes					
Lipase activity	2.20 ± 0.18 ^c	3.20 ± 0.26 ^b	4.64 ± 0.36 ^b	5.36 ± 0.25 ^a	0.01
Protease activity	7.65 ± 0.47 ^c	9.96 ± 0.28 ^b	12.07 ± 0.21 ^a	13.05 ± 0.35 ^a	0.01
Amylase activity	12.83 ± 0.55 ^c	16.07 ± 0.78 ^b	16.86 ± 0.85 ^b	21.13 ± 0.64 ^a	0.04
*One-way ANOVA showed the different superscript letters between OLFE-fortified diets are significantly different ($P < 0.05$).					

The current study enhances the knowledge of the possible applications of medicinal herbs in fish farming for cadmium exposure, immunological stimulation, and growth promotion. The findings of this study show that adding *O. littoralis* fruits to tilapia diets can enhance fish growth and feed efficiency. Due to the unique nutritional profile of the added OLFE-fortified diets, which are high in dietary fibers, carbohydrates, and amino acids, the advantage of supplemented feed might be attributed to several bioactive ingredients, such as triterpenoid glycosides and saponins, which have been shown to enhance Nile tilapia

growth and feed utilization significantly. As growth and immunological enhancers, phyto-additives, sometimes referred to as herbal additives or phyto-feed additives, are thought to be among the most favored alternatives (Salaheen et al. 2015; Mahrose et al. 2019; Hassan et al. 2019). Antioxidants, minerals, vitamins, amino acids, fruit oils, and bioactive substances are all abundant in prickly pears (Feugang et al. 2006; Bhatt & Nagar 2013; Osuna-Martinez et al. 2014). Previous research showed that prickly pears, which are members of the Cactaceae family, promoted antioxidant properties and protective capability via a variety of components, including phenolic molecules, vitamins C and E, as well as other non-nutritional components (Ramadan & Mörsel 2003; Yahia & Mondragon-Jacobo 2011). Because the primary flavonoids were separated, phenolic compounds from prickly pears were identified as antioxidative agents (Feugang et al. 2006; Saih et al. 2017; Mahrose 2021).

Table 3 depicts the levels of digestive enzymes in the intestine of tilapia fed on OLFE-fortified diets (OLFE) for sixty days. ANOVA revealed the highest amounts of lipase, amylase, and protease were seen in the OLFE-2 and OLFE-3 treatments. The improvement in growth may be attributed to the enhancement in digestion and absorption of OLFE-fortified diets, leading to improved nutrient utilization. This evidence was supported by the significant improvement in the activities of digestive enzymes, amylase, lipase, and protease, as follows: OLFE-0 > OLFE-1 > OLFE-2 > OLFE-3 treatments ($P < 0.05$) were observed. The application of medicinal plants has become increasingly popular in aquaculture in recent years because of their health-positive aspects and unique features (Abd-Elaziz et al. 2023). Other dietary supplementations (*Lactobacillus helveticus*, *Gum Arabic*) enhanced the digestive enzyme activity of carp fish (Yousefi et al. 2023b). Also, supplementation with savory essential oils (*Satureja hortensis*) in fish diets can enhance humoral immunological variables, blood protein levels, and intestinal digesting activity, as well as additionally encourage growth (Ghafarifarsani et al. 2023).

Hematological alternations of tilapia

In fish farming, studying the hematological indices is essential for estimating aquatic species' health conditions, physiological functions, and nutritional status (Fazio 2019). The hematological indices (erythrocytes, PCV, MCH, Hb, MCHC, MCV, and leucocytes) of Tilapia fed on OLFE-fortified diets for sixty days in both pre-cadmium and post-cadmium of exposure to the sublethal effects of cadmium are represented in Table 4. Before the cadmium toxicity, the levels of erythrocytes, PCV, MCH, Hb, MCHC, and MCV in tilapia fed with OLFE-fortified diets were significantly improved compared to control samples (without OLFE-fortified diets). Post-cadmium toxicity, their levels declined significantly in all OLFE-fortified diets of tilapia, and the control diet group exhibited minimal values. On the other hand, increasing the level of OLFE in the tilapia diet showed improvements in the activities of erythrocytes, PCV, MCH, Hb, MCHC, and MCV following cadmium toxicity. However, leucocyte levels were significantly higher after cadmium exposure in all treatments, whereas the highest increment levels were detected in the OLFE-0% group and the lowest increment levels in the higher OLFE-1% group. Erythrocyte counts declined in aquatic species, possibly due to anemia leading to inhibition of erythropoietin after cadmium toxicity. Abdel-Tawwab et al. (2024) and Yang et al. (2023) mentioned a significant reduction in the erythrocyte count of tilapia after cadmium toxicity. However, the elevation in leukocyte cadmium toxicity may be due

to increased lymphopoiesis and improved lymphocyte elevation from lymphoid tissues. Similar findings were recorded by Zhai et al. (2017) and Abdelzaher et al. (2022b).

Table 4

Hematological indices of tilapia fed OLFE-fortified diets for sixty days (pre-cadmium) and after exposure to sublethal effects of cadmium (post-cadmium).

Hematological indices								
		RBC ($\times 10^6$ cell / mm ³)	WBC ($\times 10^3$ cell / mm ³)	Hb (g/dl)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (%)
Pre-cadmium	OLFE-0%	2.20 $\pm$ 0.13 ^{bA}	25.37 $\pm$ 0.28 ^{aB}	7.28 $\pm$ 0.14 ^{cA}	24.36 $\pm$ 0.39 ^{cA}	110.64 $\pm$ 1.04 ^{bA}	33.06 $\pm$ 0.78 ^{cA}	29.89 $\pm$ 0.46 ^{bA}
	OLFE-0.5%	2.33 $\pm$ 0.09 ^{aA}	23.52 $\pm$ 0.71 ^{bB}	8.09 $\pm$ 0.14 ^{bA}	27.27 $\pm$ 0.12 ^{bA}	116.87 $\pm$ 1.27 ^{aB}	34.67 $\pm$ 0.18 ^{bA}	29.67 $\pm$ 0.27 ^b
	OLFE-1%	2.41 $\pm$ 0.12 ^{aA}	21.81 $\pm$ 0.24 ^{cB}	8.87 $\pm$ 0.08 ^{bA}	26.94 $\pm$ 0.13 ^{bA}	111.78 $\pm$ 1.61 ^{bB}	36.80 $\pm$ 0.31 ^a	32.93 $\pm$ 0.33 ^{aA}
	OLFE-2%	2.51 $\pm$ 0.08 ^{aA}	21.01 $\pm$ 0.31 ^{cB}	9.16 $\pm$ 0.20 ^{aA}	28.23 $\pm$ 0.10 ^{aA}	112.46 $\pm$ 2.42 ^b	36.48 $\pm$ 0.74 ^{aA}	32.44 $\pm$ 0.06 ^{aA}
Post-cadmium	OLFE-0%	1.95 $\pm$ 0.12 ^{dB}	28.53 $\pm$ 0.53 ^{aA}	5.94 $\pm$ 0.44 ^{cB}	20.91 $\pm$ 0.49 ^{cB}	107.19 $\pm$ 1.72 ^{cB}	30.45 $\pm$ 0.57 ^{cB}	28.41 $\pm$ 0.60 ^{bB}
	OLFE-0.5%	2.13 $\pm$ 0.09 ^{cB}	25.35 $\pm$ 0.62 ^{bA}	7.13 $\pm$ 0.04 ^{bB}	24.36 $\pm$ 0.39 ^{bB}	114.37 $\pm$ 1.26 ^{aA}	33.46 $\pm$ 0.44 ^{bB}	29.25 $\pm$ 0.37 ^b
	OLFE-1%	2.21 $\pm$ 0.07 ^{bB}	22.38 $\pm$ 0.68 ^{cA}	8.03 $\pm$ 0.18 ^{aB}	25.36 $\pm$ 0.30 ^{aB}	114.75 $\pm$ 0.48 ^{aA}	36.33 $\pm$ 0.36 ^a	31.66 $\pm$ 0.39 ^{aB}
	OLFE-2%	2.38 $\pm$ 0.11 ^{aB}	21.94 $\pm$ 0.56 ^{cA}	8.45 $\pm$ 0.13 ^{aB}	26.84 $\pm$ 0.08 ^{aB}	112.77 $\pm$ 1.55 ^b	35.50 $\pm$ 0.40 ^{aB}	31.48 $\pm$ 0.27 ^{aB}
Two-way ANOVA	Pre-Cd	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Post-Cd	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-Cd*Post-Cd	0.032	0.012	0.02	0.012	0.022	0.02	0.02

*Data (means $\pm$ S.E., n = 5) showed means with different small letters in the same phase revealed significant variations (ANOVA, P < 0.05) while columns with different large letters in the same diets (in both pre-cadmium and post-cadmium) revealed significant variations (T-test, P < 0.05).

The present study found significant increases in the PCV, erythrocytes, Hb, and MCH in all diet groups with OLFE supplementation compared to the OLFE-0% groups ($P < 0.05$). Supplementing diets with *O. littoralis* fruits may also improve hematological measurements, primarily indicating the fish's health and stress levels. Similar reporting by Osman et al. (2018), Fazio (2019), and Abdelzaher et al. (2022b) recorded that the hematological measurements improved after the aquatic organisms were fed a diet supplemented with herbal additives. Erythrocytes, PCV, and Hb in all tilapias of OLFE diets increased significantly compared to the group without OLFE diets (control), suggesting protective ability and an immunostimulant of OLFE against toxins. The bioactive substances (saponins, tannins, flavonoids, terpenoids, phenols, and glycosides) that have been identified as immunostimulants may be the cause of the improvement in tilapia hematological indicators (Feugang et al. 2006; Bhatt and Nagar 2013 & Osuna Martinez et al. 2014). In Nile tilapia, supplementing the diet with OLFE was reported to enhance hematological indexes, promoting Hb. This effect was also explained by bioactive components like those identified in the current study (Goda 2008). Numerous medicinal herbs are abundant in secondary metabolites, which have been linked to immune-modulating effects in stressful situations, according to Hoseinifar et al. (2020) and Gehad et al. (2023).

Biochemical alternations of tilapia

The alterations of biochemical indices in tilapia blood were documented following the toxin stress, and the blood tests serve as an effective and precise way to assess the species' well-being (Shin et al. 2016; Abbas et al. 2023). The biochemical indices (lipids, cortisol, protein, glucose, ASAT, creatinine, and ALAT) of Tilapia fed on OLFE-fortified diets for sixty days in both pre-cadmium and post-cadmium exposure to the sublethal effects of cadmium are represented in Table 5. Before the cadmium toxicity, the levels of lipids, cortisol, protein, glucose, ASAT, creatinine, and ALAT in tilapia fed with OLFE-fortified diets were significantly declined compared to control samples (without OLFE-fortified diets). Post-cadmium toxicity, their levels increased significantly in all OLFE-fortified diets of tilapia, and the control diet group exhibited maximal values. On the other hand, increasing the level of OLFE in the tilapia diet showed an improved reduction rate in the activities of lipids, cortisol, protein, glucose, ASAT, creatinine, and ALAT following cadmium toxicity. The ALAT activity in tilapia exposed to cadmium toxicity may be attributed to providing some degree of liver necrosis. Ye et al. (2011) reported that the liver enzymes ASAT and ALAT, which convert alpha-amino acids to alpha-keto acids, frequently escape into the circulatory system at high levels when hepatocytes are damaged. However, OLFE-fortified diets reduce liver enzyme production in the bloodstream because of the flavonoid and phenolic groups present, which act as hepatoprotective. Previous research has demonstrated that cadmium toxicity is linked to organ damage and elevated enzyme activity in some aquatic organisms. Similar findings were made by Yang et al. (2023) and Abdel-Tawwab et al. (2024). Increased blood cortisol has been associated with physiological stress in fish exposed to cadmium toxicity; this has been observed in different cultivated fish (Abdelzaher et al. 2022 & Abdel-Tawwab et al., 2024). Increased glucose levels (hyperglycemia) may be induced by cortisol activation to provide the necessary energy in stressful situations (Elbialy et al. 2021).

Table 5

Biochemical indices of tilapia fed OLFE-fortified diets for sixty days (pre-cadmium) and after exposure to sublethal effects of cadmium (post-cadmium).

Biochemical indices								
		Cortisol (ng/ml)	Glucose (mg/dl)	Protein (g/dl)	Lipids (g/dl)	Creatinine (mg/dl)	ASAT (U/L)	ALAT (U/L)
Pre-cadmium	OLFE-0%	3.08 ± 0.13 ^{bB}	88.50 ± 0.48 ^{aB}	2.99 ± 0.06 ^{aB}	2.04 ± 0.85 ^{aB}	0.93 ± 0.04 ^{aB}	93.45 ± 0.57 ^{aB}	28.39 ± 0.71 ^{aB}
	OLFE-0.5%	2.86 ± 0.08 ^{aB}	75.72 ± 0.31 ^{bB}	2.28 ± 0.18 ^{aB}	1.81 ± 0.36 ^{bB}	0.62 ± 0.06 ^{bB}	79.32 ± 0.82 ^{bB}	20.79 ± 0.49 ^{bB}
	OLFE-1%	2.54 ± 0.02 ^{aB}	71.62 ± 0.35 ^{cB}	2.07 ± 0.02 ^{bB}	1.69 ± 0.25 ^{bB}	0.52 ± 0.05 ^{bB}	73.90 ± 0.24 ^{bB}	16.31 ± 1.27 ^{bB}
	OLFE-2%	2.40 ± 0.09 ^{aB}	67.08 ± 0.55 ^{cB}	1.79 ± 0.09 ^{cB}	1.34 ± 0.19 ^{cB}	0.40 ± 0.07 ^{cB}	63.32 ± 0.62 ^{cB}	13.40 ± 0.23 ^{cB}
Post-cadmium	OLFE-0%	6.11 ± 0.17 ^{aA}	203.75 ± 1.49 ^{aA}	4.84 ± 0.24 ^{aA}	2.69 ± 0.44 ^{aA}	1.52 ± 0.11 ^{aA}	123.95 ± 0.53 ^{aA}	41.87 ± 0.49 ^{aA}
	OLFE-0.5%	3.31 ± 0.25 ^{bA}	143.98 ± 1.00 ^{bA}	3.08 ± 0.12 ^{bA}	2.16 ± 0.63 ^{bA}	1.23 ± 0.10 ^{bA}	90.04 ± 0.98 ^{bA}	28.46 ± 0.65 ^{bA}
	OLFE-1%	3.01 ± 0.23 ^{bA}	104.66 ± 0.79 ^{cA}	2.45 ± 0.13 ^{cA}	1.91 ± 0.38 ^{cA}	1.08 ± 0.08 ^{cA}	86.75 ± 0.66 ^{bA}	20.49 ± 0.45 ^{cA}
	OLFE-2%	2.81 ± 0.11 ^{cA}	97.64 ± 0.36 ^{cA}	2.11 ± 0.09 ^{cA}	1.76 ± 0.13 ^{cA}	0.98 ± 0.11 ^{cA}	73.27 ± 0.67 ^{cA}	17.84 ± 0.34 ^{cA}
Two-way ANOVA	Pre-Cd	0.04	0.03	0.04	0.03	0.03	0.03	0.03
	Post-Cd	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	Pre-Cd*Post-Cd	0.024	0.02	0.03	0.01	0.01	0.01	0.01

* (ASAT) Alanine aminotransferase. (ALAT) Aspartate aminotransferase. Data (means ± S.E., n = 5) showed significant differences in bars with different small letters in the same phase (ANOVA, $P < 0.05$) and different large letters in the same diets (pre-cadmium and post-cadmium, T-test, $P < 0.05$).

Conversely, the lower blood glucose levels observed in the OLFE-fortified groups under pre-cadmium and post-cadmium exposures in comparison to the OLFE-0% (control group) could potentially be attributed to

O. littoralis's hypoglycemic effect, which is mediated by the presence of bioactive ingredients, specifically phenols, flavonoids, and saponins. Parwata et al. (2018) revealed that flavonoids and phenolic constituents can reduce concentrations of glucose in the blood because of their strong antidiabetic effects. Furthermore, among the many biological consequences of saponins, a type of phytochemical, is the reduction of glucose levels and inhibition of the enzymes that change disaccharides into simple carbohydrates (Oishi et al. 2007).

Protein levels in fish blood may indicate the health of the fish (Ngugi et al. 2017). Likewise, fish-fed diets supplemented with immune stimulants have greater serum levels of total protein, which are linked to an innate immune response (Rudneva and Koverchina 2011). Therefore, the study's findings that fish fed OLFE-fortified diets had significantly higher total protein in both pre-cadmium and post-cadmium exposure imply that OLFE-based diets boost tilapia fish's innate immunity. These observations are consistent with Goda (2008), Sonmez et al. (2015), and Yang et al. (2023), who reported that herbal plants used as a supplemented diet increased the serum protein levels in fish. After cadmium toxicity, all OLFE-fortified diets effectively reduced all biochemical parameters (lipids, cortisol, protein, glucose, ASAT, creatinine, and ALAT). Still, the most effective level was in the OLFE-2% groups. In line with current findings, the use of herbal plant supplements in the diet significantly lowered the increase of cadmium toxicity in all biochemical variables (lipids, cortisol, protein, glucose, ASAT, creatinine, and ALAT), as stated by Arup and Patra (2011), Zhai et al. (2017), and Abdelzaher et al. (2022).

Antioxidative biomarkers of Nile tilapia

Herbal supplements have benefits including promoting optimal oxidative environments for living organisms and reducing oxidative stress through free radical inhibition and reduction in antioxidant enzymes (Sonmez et al. 2015; Bilen et al. 2020; Elbesthi et al. 2020). GPx, CAT, and SOD are examples of antioxidant enzymes that are the initial line of protection against oxidative stress (Farombi et al. 2007), and their levels are provided as markers that indicate an organism's health (Ding et al. 2015). As a biomarker for the detrimental effects of reactive oxygen species (ROS), MDA results from lipid peroxidation (Lushchak 2011). The main defensive mechanisms against oxidative stress in fish tissue are antioxidant defense systems, which can protect against cell damage from an overabundance of reactive oxygen molecules (Hoseinifar et al. 2021). The first line of the immune response, which is constructed from the activities of CAT (catalase), SOD (superoxide dismutase), and GPX (glutathione peroxidase), is essential to the overall defensive mechanisms and techniques in biological processes. Many types of enzymes, including SOD, CAT, and GPX, are involved in enzymatic antioxidant processes. These mechanisms enable the fish's body to scavenge free radicals, a crucial defensive function (Ighodaro & Akinloye 2018).

The antioxidant biomarkers (SOD, CAT, and GPX activities) and MDA of tilapia fish in both pre-cadmium and post-cadmium exposure to cadmium toxicity are represented in Fig. 1. Before the cadmium toxicity, the activities of CAT, SOD, and GPX in tilapia fed with OLFE-fortified diets were significantly improved compared to control samples (without OLFE-fortified diets). Post-cadmium toxicity, their levels increased significantly in all OLFE-fortified diets of tilapia, and the control diet group exhibited minimal values. On

the other hand, increasing the level of OLFE in the tilapia diet showed improvement in the activities of CAT, SOD, and GPX following cadmium toxicity. A similar finding was revealed by Yang et al. (2023), who stated that there has been observed to be an improvement in SOD activity. Also, Hassan et al. (2022) and Abdel-Tawwab et al. (2024) reported that fish subjected to cadmium toxicity seem to cause increases in the production of hydrogen peroxide, which enhances CAT activity.

Malondialdehyde functions as a stable indicator of lipid peroxidation and protein oxidation, and its concentrations may suggest how vulnerable fish body cells are to free radical damage. Lipid peroxidation increases fish's MDA levels under oxidative stress (Ciftci et al. 2011; Taheri Mirghaed et al. 2019b). MDA activities were significantly increased after cadmium exposure in all groups, whereas the maximum increment rate was detected in the OLFE-0% group and the lowest increment levels in the higher OLFE-1% group. In line with the current study's outcomes, diets enriched with OLFE considerably reduced the levels of MDA, suggesting that these diets may offer some protection against oxidative damage. Similarly, grass carp (*Ctenopharyngodon idella*) fed on diets supplemented with SB had higher antioxidative capacities due to increased SOD and GPx enzymes and decreased MDA amounts (Wu et al. 2018).

In the current study, OLFE-fortified diets significantly decreased MDA and increased concentrations of enzymatic antioxidants compared to the OLFE-0% group in both pre-cadmium and post-cadmium exposure. The bioactive substances found in OLFE, such as phenols, flavonoids, tannins, and terpenoids, which are important for their antioxidant and lipid peroxidation capabilities, can also help to explain these results by Rodriguez-Mateos et al. (2014), Mata et al. (2016), Andreu et al. (2018), and Berrabah et al. (2019). According to Yeddes et al. (2013), these phytochemicals are superior singlet oxygen quenchers, metal chelators, reducing agents, and hydrogen donors. The OLFE-fortified food supplement dramatically improved the antioxidative enzymes CAT, SOD, and GPx (Moussa-Ayoub et al. 2014; Mena et al. 2018; Albuquerque et al. 2020). This finding is in accord with Jia et al. (2017) and Li et al. (2020), who mentioned that increased activity of antioxidant enzymes helps prevent the production of free radicals and lessen the damage caused by lipidic superoxide in tilapia fish. The high degree of antioxidant enzyme activity may prevent oxidative damage.

Immunological indices of Nile tilapia

Innate immunological responses, such as complement C3 and lysozyme activity, control the body's overall immunity in the face of infection or stress. Despite being a component of the adaptive immune system, aquatic organisms' immunological state may be determined by their serum Ig level (Xu et al. 2021; Yousefi et al. 2023). Research indicates that adding herbal supplements to the fish diet might enhance the activities of total Ig lysozyme and enhance the effects of complement C3, both of which strengthen fish's resistance to the following stressors (Nya and Austin 2011; Talpur and Ikhwanuddin 2013; Taheri Mirghaed et al. 2019b; Yang et al. 2023; Abdel-Tawwab et al. 2024).

The immunological parameters, complement C3 lysozyme, and total Ig activities of tilapia in both pre-cadmium and post-cadmium exposure to cadmium toxicity are represented in Fig. 2. Before the cadmium toxicity, the activities of complement C3, lysozyme, and total Ig in tilapia fed with OLFE-fortified diets

were significantly improved compared to control samples (without OLFE-fortified diets). Post-cadmium toxicity, their levels declined significantly in all OLFE-fortified diets of tilapia, and the control diet group exhibited minimal values. On the other hand, increasing the level of OLFE in the tilapia diet showed improvement in the activities of complement C3, lysozyme, and total Ig following cadmium toxicity. Thus, the current study suggests that OLFE stimulates the immune system in tilapia fish, as seen by increased levels of serum-soluble immune components. Furthermore, fish exposed to cadmium toxicity have been reported to have immunosuppressive responses, consistent with the current findings (Yang et al. 2023). The current findings indicate that dietary OLFE-fortified diets help tilapia fish by reducing the immunosuppression brought on by exposure to cadmium toxicity. Comparably, it has been discovered that dietary phytochemicals might lessen the negative effects of cadmium toxicity on fish species' serum total Ig, lysozyme, and complement C3 (Abdel-Tawwab et al. 2024).

These results corroborate those of other studies looking into using plant chemical extracts to boost immunity in farmed fish. These studies have found that a variety of bioactive compounds and phytochemicals, such as pigments, terpenoids, alkaloids, steroids, and phenolics, stimulate various biological processes in farmed fish, including complement system activation, phagocytic activation, immunostimulant, and anti-stress responses (Citarasu 2010; Chakraborty et al. 2014; Radwan et al. 2022). Gao et al. (2020), Liu et al. (2021), Esam et al. (2022), and Guo et al. (2023) found that ecological toxins may affect the activities of IgM, complement C3, complement C4, and lysozyme, which may affect fish species' immunological characteristics. In our investigation, tilapia's total Ig, lysozyme, and complement C3 activities decreased following cadmium toxicity transport, suggesting a persistent deterioration in immune function. Additionally, Abdel-Tawwab et al. (2024) observed that during cadmium toxicity, fish displayed a considerable drop in complement C4, complement C3, IgM levels, and LZM activity.

Conclusion

The provided results provide a complete picture of how well dietary *O. littoralis* fruits protect tilapia fish from the harmful effects of cadmium. The exposure of tilapia fish to cadmium toxicity resulted in significant reductions in hematological variables, significant changes in biochemical indexes, and a fall in immunological and antioxidative biomarkers, contingent on the quantities of *O. littoralis* fruits provided with fed diets. Based on the study's findings, tilapia diets supplemented with different levels of OLFE may boost the utilization of feed, growth, and stress resistance. Elevated OLFE levels have been linked to the immune system and antioxidant activation. Fish exhibited the best results in all parameters when given OLFE levels up to 2% in the present investigation. Our research demonstrates that adding *O. littoralis* fruits to aquatic animal feeds can be an environmentally friendly method of promoting sustainable aquaculture. It also reduces immunological indicators and increases antioxidant activities, which mitigate the harmful consequences of Cd toxicity.

Statements and Declarations

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Author Contributions

Mahmoud Mahrous M. Abbas: designed the research idea, Methodology, Original draft writing, and statistical analysis. **Mohamed A. Amer:** Methodology, Writing – review and editing Visualization. **Jamila S. Al Malki:** Figuration, and Tabulation Statics-formal analysis, review, and editing. **Amaal Mohammadein:** Formal analysis, Statistical analysis, Methodology, Editing. **Metwally G. Metwally:** Figuration and Tabulation, Methodology. **Rania M. Waheed:** Formal analysis, Statistical analysis, Methodology, Editing. **Said M. A. Elraey:** Formal analysis, Statistical analysis, Methodology, Editing. **Mahmoud Radwan** Sampling Methodology, Conceptualization, Editing. All authors read and approved the final manuscript.

Ethical approval The study was conducted according to the Ethics Committee of Institutional Animal Care and Use Committee guidelines, Zagazig University, Egypt (No. ZU-IACUC/1/F/66/2024).

Availability of data and materials

The data sets in this study are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

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Figures

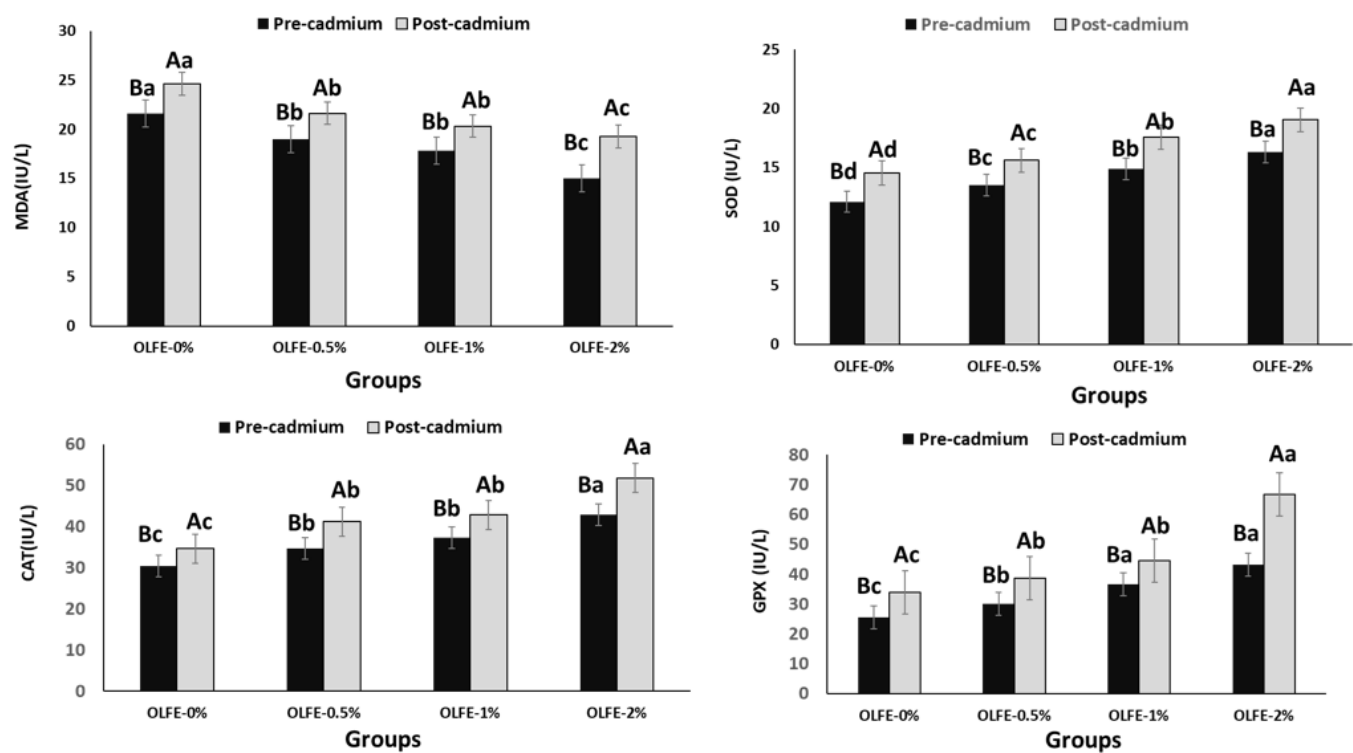


Figure 1

Variations in activities of CAT (catalase, IU/L), SOD (superoxide dismutase, IU/L), GPX, (glutathione peroxidase, IU/L), and MDA (malondialdehyde) levels in tilapia fed OLFE-fortified diets (OLFE) for sixty days (pre-cadmium) and after exposure to sublethal effects of cadmium (post-cadmium). Data (means ± S.E., n = 5) showed significant differences in bars with different small symbols in the same phase (ANOVA, $P < 0.05$) and different large symbols in the same diets (pre-cadmium and post-cadmium, T-test, $P < 0.05$).

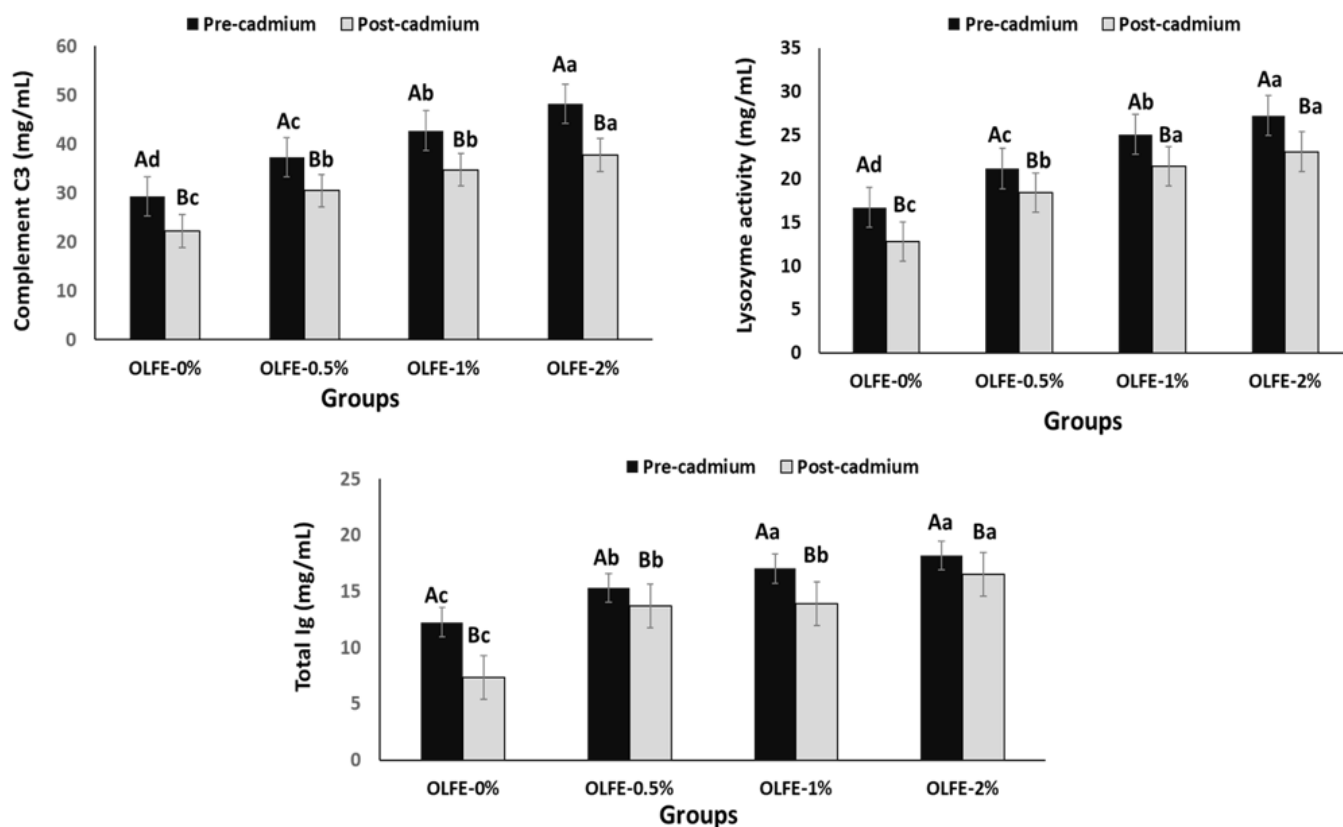


Figure 2

Changes in activities of complement C3 (mg/mL), lysozyme, and total Ig in Nile tilapia fed OLFE-fortified diets for sixty days (pre-cadmium) and after exposure to sublethal effects of cadmium (post-cadmium). Data (means $\pm$ S.E., $n = 5$) showed significant differences in bars with different small symbols in the same phase (ANOVA, $P < 0.05$) and different large symbols in the same diets (pre-cadmium and post-cadmium, T-test, $P < 0.05$).