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Impact of Moringa (*Moringa oleifera*) Leaves on the Health Status of Nile Tilapia (*Oreochromis niloticus*) Exposed to Cadmium Toxicity

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Abstract

MEDICINAL plants have recently gained more attention in sustaining fish health. This study focuses on using moringa leaves (crude and extract) as a feed additive to enhance Nile tilapia (*Oreochromis niloticus*) health including growth, immunity, and antioxidant enzymes in the presence of cadmium toxicity. Fish were divided into six groups: two control groups (one negative, (C) and one positive exposed to cadmium chloride (Cd)). The other four groups were exposed to cadmium chloride while fed with 1% (T1) &0.5% (T2) of crude moringa leaves and with 1% (T3) &0.5% (T4) of moringa leaves extract. After 30 days, growth, hematological, biochemical, immunological parameters, antioxidant enzymes, and cadmium residues in different tissues were assessed. The results of Cd group showed a significant reduction in weight gain (WG), specific growth rate (SGR), white blood cell count (WBC), lymphocyte %, and lysozyme activity. Also, it showed a significant increase in protein, albumin, alkaline phosphatase, triglycerides, glucose, cholesterol, catalase (CAT), and glutathione-S-transferase (GST) levels. Crude moringa groups revealed a significant enhancement in WBC, lymphocyte, protein, albumin, globulin, and lysozyme activity and a decrease in CAT and GST values compared to Cd group. Also, they recorded an increase in WG and SGR compared to Cd group. The present study showed that moringa triggers cadmium accumulation in liver and gills however, it was significantly lower in gonads and muscles of moringa-treated groups. The study concludes that crude moringa leaves, especially at low concentrations (0.5%), are safe as feed additives to enhance overall health, growth, and immunity in Nile tilapia, and mitigate the toxic effects of cadmium.

Keywords: Moringa leaves, Nile tilapia, Growth performance, Immune status, Antioxidants, Cadmium residues.

Introduction

Growing and sustaining aquaculture represents one of the main and vital aims in facing the challenges of protein deficiency and high costs in developing countries. However the widespread problem of water pollution is jeopardizing fish health, it positively correlates with fish growth, immunity, reproduction, and general health [1]. Water pollutants include any harmful substance that contaminates the water body such as oil, sewage, radioactive, and heavy metals. The virulence of heavy metals stems from their non-degradable nature and their ability to be bioaccumulated in vital fish tissues [1]. Cadmium (Cd) is an inorganic metal that can enter water bodies as a result of industry activities such as batteries, painting, plastic, and metal plating [2], or through sewage sludge and phosphate fertilizers [3]. Cd represents one of the most hazardous heavy metals that affect and retard many functions in fish as osmoregulation, reproduction, and growth [4]. Previous studies reported harmful effects of Cd on fish like reduction in weight gain of *Sebastes schlegeli* [5] and *O. niloticus* [6], and dangerous changes in tissue histopathology [7,8]. Cd induces redox reactions and generates reactive oxygen species (ROS) [7,9].

Since plants are environmentally friendly and have low cost, low generation of by-products, and biodegradability, they represent a promising solution to aquaculture challenges including the water pollution problem [10]. Moringa (*Moringa oleifera*) belongs to the family Moringaceaem, which is naturalized in many African countries, known as ‘‘the tree of life”. It is widely regarded as one of the most valuable trees globally due to its abundance of bioactive compounds, that exhibit great economic

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importance in food and medicinal applications [11,12]. Moringa has many nutritive values, the crude protein content in leaves reaches 260g/Kg dry weight, has phytochemicals and different essential amino acids, so it is recognized as a food supplement for humans and animals [13]. Moringa leaves have a lot of nutritional constituents such as carbohydrates, protein, fiber, fat, ash, and moisture. Also, it contains some important vitamins and minerals; calcium, phosphorus, and iron [14].

Dietary supplementation with a low dose of moringa improved the growth performance of catfish, however, the increased dose of moringa reduced the growth and decreased the digestibility and palatability [15]. Moringa stimulates the growth performance and the immune system of Nile tilapia and increases its resistance against *Aeromonas hydrophila* [14,16]. Moringa potentiates the antioxidant status of fish by increasing superoxide dismutase (SOD) and catalase (CAT) in the gills, liver, and kidney [17,18]. Moreover, moringa has been used as a natural coagulant in wastewater treatments [10]. Several authors revealed that moringa seeds biomass has sufficient active binding sites for metal ions; so it can remove heavy metals from water solutions [19 - 21]**.**

The current investigation was concerned with studying the effect of using moringa leaves either as crude or as an extract on the health status of Nile tilapia exposed to cadmium by studying their effects on growth, hematological, biochemical, immunological, and antioxidant parameters. In addition to evaluating whether moringa leaves have the power to mitigate cadmium toxicity and reduce their accumulation in body organs.

Material and Methods

Ethical approval

The study was approved by the Institutional Animal Care and Use Committee, National Research Centre, Egypt.

Moringa extract and diet preparations

Crushed leaves of moringa (*M. oleifera*) were purchased from the Moringa products outlet at the National Research Centre, Cairo, Egypt. The extract was prepared by immersing 500 g of crushed moringa leaves in twice the volume of absolute ethyl alcohol within a stoppered container. Then the extract was filtered, and evaporated by a rotary evaporator before being left for complete dryness and weighed [22]. A commercial pellet diet (Skretting Egypt, Hendrix) (floating 35% protein) was crushed, mixed with 1% (T1) and 0.5% (T2) of crude moringa leaves and with 1% (T3) and 0.5% (T4) of moringa leaves extract. The mixture was moistened with water, then reshaped into pellets and allowed to air dry. Once dried, the pellets were stored in the refrigerator until needed.

Experimental design and sampling

Two hundred and seventy Nile Tilapia fish (*O. niloticus*), of average weight 30 ±5 g were obtained from a fish farm at Kafr El Sheikh. The fish were given a two-week acclimatization period in aerated, free-flowing freshwater. Fish were divided into six groups, each group consisting of 45 fish (15 per replicate). Fish were fed on 2% of body weight daily. The first 2 groups were fed commercial fish food without additives (35% protein, 5.8% fats, and 3.5% fibers), one was considered a negative control (C) and the second was exposed to cadmium chloride (Rasayan Laboratory finne-chem LTd., Haryanan, India) and considered a positive control (Cd). The other four groups were exposed to cadmium chloride and fed with 1% (T1) and 0.5% (T2) of crude moringa leaves and with 1% (T3) and 0.5% (T4) of moringa leaves extract. Cadmium chloride was in a concentration of 1.5 mg Cd/l of $CdCl_2$, which represents 1/10 LC_{50} of Cd according to Garcia-Santos *et al*. [23]. After 30 days of feeding, blood and tissue samples were collected.

Fish were anesthetized by clove oil (0.5ml/l). Blood samples were obtained from the caudal vein of fish using a syringe, and divided into two portions. One portion was collected in anticoagulant EDTA tubes for hematological assays, while the other portion was collected in Vacuettes tubes, centrifuged for 15 minutes at 3000 rpm, and the serum was collected before being stored at -20º C until used in biochemical and immunological assays.

Growth performance

Total length, standard length, liver weight, gonads weight, and total weight of fish from each treatment were recorded at the beginning and the end of the experiment. Growth indices (weight gain (WG), specific growth rate (SGR), hepato-somatic index (HSI), gonado-somatic index (GSI) and the condition factor (CF)) were determined by the following formula [24].

WG = final weight – initial weight
SGR = 100* (final weight – initial weight)/ feeding days
HSI = (liver weight/total weight) x100
GSI = (Gonads weight/total weight) x100
CF = (total weight/total length^3) x100

Haematological assays

Red blood cells (RBC) and white blood cells (WBC) were enumerated utilizing Neubauer hemocytometer [25]. The differential leukocytic count was documented following staining with Giemsa stain. Hematocrit (Hct%) was measured using heparinized capillary tubes, centrifuged in a hematocrit centrifuge. Hemoglobin (Hb) was evaluated according to the cyanmethemoglobin method [26].

Immunological parameters

Antiproteases activity

The activity was determined according to the protocol by Lange *et al*. [27]. Briefly, 20 µl of a trypsin solution (Sigma-aldrich) (5 mg/ml) and 20 µl of serum were incubated for 10 minutes at 22˚C. Then, 200 µl of 0.1 M PBS and 250 µl of a 2% azocasein solution (20 mg/ml PBS) were added to the mixture, then incubated for one hour at 22˚C. The reaction was stopped with 500 µl of 10% (v/v) trichloroacetic acid (TCA), followed by a 30-minute. The mixture was centrifuged at 6000 x g for 5 minutes. Then, 100 µl of the supernatant and 100 µl of 1 N NaOH were placed in a 96-well flat-bottom plate. Absorbance was read at 410 nm using an ELISA reader. A positive control (100%) was prepared by substituting serum with buffer. The percentage inhibition of trypsin activity was determined by comparing it to the positive control.

Lysozyme activity

Lysozyme activity was evaluated following the method outlined by Parry *et al.* [28]. In brief, 60 µL of serum was combined to 2 mL of a suspension of Micrococcus lysodeikticus (Sigma-aldrich) (0.2 mg/ml) in 0.05 M PBS. The absorbance was recorded at 530 nm using a spectrophotometer at 30 seconds and after 4 min. and 30 seconds. A unit of lysozyme activity was defined as the quantity of sample that induces a decrease of 0.001 absorbance units per minute.

Total protein, albumin and globulin

Total protein and albumin were estimated using commercial biochemical kits (Bio-diagnostics, Egypt) [29-30]. Globulin was evaluated by subtracting albumin concentration from total protein. The colorimetric reaction was measured using an Agilent Cary UV-Vis spectrophotometer.

Biochemical assays

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), uric acid and creatinine, triglycerides, cholesterol, and glucose were evaluated by biochemical kits (Bio-diagnostics, Egypt) using a spectrophotometer [30-35].

Determination of antioxidant enzymes

Liver samples were collected and immediately immersed in liquid nitrogen before being stored at -80°C. About 0.5 g of liver sample was then homogenized in a KCl-HEPES buffer containing 0.15M KCl and 0.02M HEPES at pH 7.5 and centrifuged at 9000 g for 30 min at 4°C. The collected supernatants (S9 fraction) were used for antioxidant enzymes (catalase (CAT), peroxidase (POD), glutathione-S-transferase (GST), superoxide dismutase (SOD)) [36]. The protein content of S-9 fraction was measured using the method described by Lowry *et al.* [37] using BSA as a standard protein. CAT activity was evaluated following the procedure outlined by Aebi [38]. Briefly, a mixture composed of 50 mM hydrogen peroxide (H_2O_2) in 50 mM phosphate buffer (pH 7.0), with a final volume of 1 ml. The reaction commenced upon addition of the liver extract, and the reduction in hydrogen peroxide absorbance was tracked at 240 nm. POD activity was measured as described by Gülçin and Yildirim [39]. Briefly, a mixture contained one ml volume 8 µ moles of H_20_2, 60 µ moles guaiacol, 150 µ moles sodium acetate buffer, pH 5.6. The alteration in absorbance at 470 nm resulting from guaiacol oxidation was monitored every 30 seconds. GST activity was measured by the conjugation of 1mM CDNB (1-chloro-2,4-dinitrobenzene) in ethanol with 1mM GSH (reduced glutathione) in a 0.1M PBS, pH 6.5. Then he absorbance of the resulting GSH-CDNB conjugate was measured at 340 nm. The specific activity of GST was determined using a molar extinction coefficient of 9.6 mM-1cm-1, and it was represented as µmoles per minute per milligram of protein [40]. SOD activity was evaluated following the procedure outlined by Villa-Cruz *et al*. [41]. A 50 µl aliquot of the supernatant, diluted 20-fold, was combined with 1 ml of a mixture containing EDTA methionine, phosphate buffer, NBT, and riboflavin, and then incubated at 25°C for 25 minutes. The activity was read at 580 nm. One unit of enzyme activity was defined as the amount needed to inhibit the oxidation of NBT by 50%. The activity was represented as units of SOD per milligram of protein.

Determination of cadmium residues:

Residues of cadmium in muscle, liver, gills, and gonads were determined according to Riyahi [42]. Briefly, 0.5g from each tissue/5 fish were pooled and digested in 5 ml of concentrated nitric acid on a hot plate at 60^{o}C for 10 min. After getting a transparent solution (complete digestion), the final volume was adjusted to 10 ml with distilled water, and cadmium was determined by an atomic absorption spectrometry.

Statistical analysis

The results are displayed as means ± SE. Differences between the control and experimental groups were evaluated for significance using a one-way ANOVA test [43] in the SPSS (version 17.0).

Results

Growth performance

The present study revealed a decrease in WG and SGR in the positive control group (Cd) compared to the negative control group (C) (Table 1). Fish groups fed on crude moringa leaves (T1 and T2) showed an increase in WG and SGR compared to Cd group. The highest WG and SGR values were recorded in T2 group (2.37±0.21g and 0.27±0.02, respectively). On the other hand, fish

groups fed on moringa extract (T3 and T4) showed a significant decrease ($P < 0.05$) in both WG and SGR compared to other groups. HSI was significantly decreased ($P < 0.05$) in Cd group compared to C group while there was a significant increase in the T1 and T2 groups compared to Cd group. GSI and CF results showed non-significant differences between the treated groups.

Hematological parameters

Total RBC count, Hb and HCT% showed nonsignificant changes ($P > 0.05$) among the control and treated groups. Cd group showed a significant decrease ($P < 0.05$) in the total WBC count and lymphocyte % compared to the C group (Table 2). Moringa-treated groups showed a significant increase ($P < 0.05$) in WBC and lymphocyte % compared to Cd group (the highest values were recorded in T2 group). There were nonsignificant changes in neutrophils, eosinophils, and monocytes between the tested group.

Immunological parameters

The current study revealed a significant increase ($P < 0.05$) in protein and albumin concentrations in Cd group compared to C group (Table 3). Fish fed 0.5% moringa crude (T2) recorded the highest significant ($P < 0.05$) total protein and albumin values compared to the Cd and C groups. Concerning globulin values, they showed a significant increase ($P < 0.05$) in all moringa-treated groups (T1, T2, T3, and T4) compared to both C and Cd groups. Regarding lysozyme activity, Cd group showed a decrease in activity compared to C group. The highest significant lysozyme activity ($P < 0.05$) was shown in groups fed moringa crude; (T2 and T1, respectively) compared to the Cd and C groups. There were no significant changes in antiprotease activity in Cd and C groups. Again, the highest significant antiprotease activity ($P <0.05$) was recorded in T2 and T1 groups, respectively, compared to other moringa-treated groups.

Biochemical parameters

The present study revealed nonsignificant changes in ALT, AST, uric acid, and creatinine values between the controls and treated groups (Table 4). On the other hand, triglycerides and ALP in Cd group and all moringa-treated groups (T1, T2, T3, and T4) showed significantly increased values ($P < 0.05$) compared to the C group. Concerning glucose and cholesterol values, they showed a significant increase ($P < 0.05$) in Cd group compared to C group. T2 and T4 groups showed the lowest significant glucose values compared Cd group ($P < 0.05$) in glucose was in T1 compared to C group. In cholesterol, the highest significant value ($P < 0.05$) comparing to Cd and C groups was recorded in T2 followed by T1and T3, respectively.

Hepatic antioxidant activities

All Hepatic antioxidant activities (Table 5) were increased in Cd group compared to C group and a significant difference was observed in only CAT and GST ($P < 0.05$). T1 group recorded the lowest value of CAT compared to other groups and showed a significant difference ($P < 0.05$) with Cd. The highest significant value ($P < 0.05$) of CAT was showed in T3 group compared to other groups. Concerning GST values in moringa-treated groups, the lowest value was recorded in T2 group with significant difference with Cd group. A significant increase ($P < 0.05$) in SOD and POX values were recorded in T2 group compared to both C and Cd groups.

Cadmium residues

Generally, cadmium concentrations in liver, gill, gonads, and muscle tissues were significantly increased ($P < 0.05$) in Cd group compared to C group (Table 6). Concerning moringa-treated groups, cadmium concentrations in the liver showed a significant increase in their values ($P < 0.05$) compared to Cd and C groups. While cadmium concentrations in the gills showed a significant increase in T2, T3 and T4 groups ($P < 0.05$) compared Cd, and C groups. On the other hand, cadmium concentration in gonads of moringa-treated groups was significantly decreased ($P < 0.05$) compared to Cd group. The lowest cadmium concentration in gonads was recorded in T1 group. The lowest significant cadmium concentration ($P < 0.05$) in muscles was in T2, followed by T4, compared to Cd group.

Discussion

Pollution with heavy metals threatens aquatic organisms and affects the sustainability of aquaculture. Cadmium negatively affects fish growth, immunity, physiological functions, and reproduction [44]. Using of natural plants as food additives in fish diets has received great attention due to their effective roles as growth promotors, immunostimulants, antioxidants, and inhibitors of xenobiotic side effects [45-48]. The reduction in WG, SGR, and HSI of Nile tilapia after exposure to Cd for 30 days which was reported during this study agrees with previous studies that documented the ecotoxicological effect of cadmium on the growth performance of Nile tilapia [6,49]. The increase in WG and SGR after feeding on crude moringa leaves while exposure to cadmium denotes the protective role of moringa against the side effects of cadmium. In fact moringa leaves are rich in proteins, vitamins, fatty acids, and minerals and are widely used as a food source for humans and animals [12]. The highest WG and SGR were recorded in the group fed on the lower dose (0.5%) of moringa (T2), suggesting this dose is an optimal dose for growth. Previous studies revealed that the higher dose of moringa leaves (12, 24, and 36%) depressed the growth of

Nile tilapia [50]. Also, Bbole *et al*. [51] indicated that moringa leaves meal decreased the growth of Nile tilapia at concentrations of 5, 10 and 15%. On the other hand, the decrease in WG and SGR in extract 1% and 0.5% treated groups could be attributed to the presence of high levels of antinutrient substances such as saponin and total phenolics content in moringa extract than the crude one. Saponin is known to have a detrimental effect on the growth of rainbow trout [52] and tilapia [53]. Another study on the effect of moringa leaves on catfish revealed that the concentration of dietary moringa should not exceed 100g/kg of fish to be safe for fish health [54]. The nonsignificant differences in GSI and CF values are comparable to results reported by Bbole *et al*. [51] who reported that the gonadal somatic, hepatosomatic, and cardiosomatic indices were not significantly different between moringa-treated groups and control.

Hematological analysis reflects the physiological response of fish to any environmental change. Thus it is very important to assess the suitability of any food additive on fish health. Nonsignificant results of RBC count, and Hb may indicate the safety of using moringa and it has no hazardous effect on the hematological functions of fish, this result is also similar to that of Bbole *et al*. [51] and Abd El-Gawad *et al*. [18] who found a non-significant change in the total count of RBC, and Hb content in moringa leaves dietary treated tilapia. The decreased values of WBC and lymphocyte % in the cadmium-exposed group may be attributed to the immunosuppression effect of cadmium. While their increased values when feeding on moringa leaves reflect their immunostimulant effect [16]. Enhancement of WBC count was previously reported in Nile tilapia fed on crude moringa leaves [16,18] and onion extract [49]**.**

Proteins in serum have many physiological roles in protecting fish. Specifically, acute-phase proteins contribute to protection by repairing tissue damage, eliminating microorganisms, and preventing the spread of infectious agents [55, 56]. Total protein in the blood includes two main proteins; albumin and globulin. Albumin originates from the liver and is responsible for keeping fluids inside the vessels and helping enzymes and vitamins in their circulation. Globulin originates from the liver by the immune system and play an important role in disease resistance [57]. The increased values of total protein, albumin, and globulin in the Cd group, could be explained by the effect of cadmium on inducing some antioxidant enzymes. Cadmium induces liver and kidney tissues to synthesize a low molecular weight protein (Metallothionein) which can tightly bind toxic Cd ions [44]. The significant increase in protein and globulin values in all moringa-treated groups compared to Cd and C groups indicated the immunomodulatory effect of moringa leaves to enhance immunity in fish and resist Cd dangerous effects. Such a conclusion is supported by Sherif *et al.* [58] and Elgendy *et al.* [16] who reported a significant increase in the immune parameters like; protein, albumin, and globulin of Nile tilapia after feeding with moringa leaves. The immunostimulant effect of moringa may be attributed to the presence of many active phytochemicals in moringa (polyphenols, vitamin E, A, volatile oils, and ascorbic acid), also, to the secondary metabolites of moringa (flavonoids, phenolic acids, saponins, alkaloids, carotenoids, and sterols) which stimulate the fish immune system [59,60]. Lysozyme plays a crucial role in the immune defense systems of both invertebrates and vertebrates. It represents one of the most important defense lines in fish through lytic and opsonin effects against infectious diseases [61,62]. Our results demonstrated a significant increase in lysozyme activity in crude moringa-treated groups (T1 & T2) compared to Cd and C groups. Such observation could be attributed to the effectiveness of moringa leaves crude in resisting Cd toxicity effect and enhancing the immune system. The efficiency of medical plants in enhancing the immune response to tolerate or resist toxic materials has been documented in fish. For example, mustard extract demonstrated effectiveness as an immuno-modulator to resist the severe impacts of BaP and increase the immune parameters of Nile tilapia like lysozyme activity, antiprotease activity, and total protein [46]. Also, using beetroot extract of Nile tilapia resisted the toxic effect of lead and enhanced the immune response parameters (lysozyme, total protein, and albumin) [63].

Biochemical parameters represent a good biomarker for fish health, especially when exposed to external environmental stimuli such as water pollutants or even new food additives [47]. The nonsignificant changes in liver and kidney functions in cadmium treated group are unlike to those reported formally and stated an increase in ALT and a decrease in AST and uric acid levels [47]. Such results are more or less in accordance with Liu *et al*. [44] who stated that the toxic effects of cadmium are not the same in different fish and tissues. The nonsignificant changes in liver function enzymes (ALT, AST) and kidney indices (uric acid, creatinine) in all moringa-treated groups in the present study come in contrary to some previous studies that reported an increase in ALT and AST in Nile tilapia fed on 0.25% of the extract of moringa flower [17]. Another study reported a decrease in ALT, AST, uric acid, and creatinine in Nile tilapia fed on moringa leaves up to a concentration of 1.5% [64]. Some previous studies reported an elevation in glucose and cholesterol levels after exposure to cadmium; in rainbow trout [65], and in Nile tilapia [47]. Similarly, the present study reported a significant increase in glucose, cholesterol, and ALP levels in the cadmium-treated group than the control group. The low dose of moringa extract 0.5% (T4) can alter the toxic effect of cadmium and decrease glucose, cholesterol, and

ALP levels compared to the Cd group. Similar results were reported by Abdelhiee *et al*. [66] who investigated the improvement in the biochemical parameters of Nile tilapia after being fed on aflatoxin-contaminated diets supplemented with 0.5% moringa.

Antioxidant enzymes play an important role in detoxification and removal of extra production of reactive oxygen species (ROS) which affect the fish health [67,68]. The increase in GST and CAT activities in the Cd group is due to the toxic effect of cadmium and the extra production of ROS which represent a serious hazard to fish health [7]. Similarly, Abbas *et al.* [47] reported a significant increase in CAT, GST, and SOD in Nile tilapia exposed to cadmium toxicity. Concerning moringa-treated groups, it was obvious that crude moringa leaves can mitigate the toxic effect of cadmium by decreasing CAT and GST activities. Similarly, previous investigations have stated the mitigation role of moringa against several pollutants, such as the role in decreasing the oxidative effect of copper nanoparticles in common carp [69], sodium fluoride in seabream [70], and chlorpyriphos in Nile tilapia [71]. Other plants showed protective effects against cadmium toxicity. For example, fenugreek seed extract (1% and 3%) used as a food additive in Nile tilapia diet, reduced the antioxidant alterations induced by cadmium exposure by decreasing CAT and SOD activities [47]. Also, dietary supplementation with 0.5% onion extract reduced GST and SOD in Nile tilapia exposed to cadmium toxicity [49].

Previous studies reported that some medicinal plants have an antidote effect on heavy metal poisoning through increasing elimination from the fish body via bile or urine, decreasing the absorption of pollutants, and their bioaccumulation in different fish tissues. Consequently, medicinal plants decrease the poisonous effects of heavy metals on many physiological functions [72]. The present study revealed a significant increase in cadmium concentrations in the liver and gills in moringa-treated groups. It is worth mentioning that the liver and gills are non-edible organs, have a great blood supply, and are responsible for heavy metal excretion and detoxification from the fish body [73]. Such results may be attributed to the chelating properties of moringa, and the presence of many amino acids with different functional groups, that can generate positive or negative charges to attract either cations or anions [74]. On the other hand, cadmium accumulation in muscle was decreased in the group receiving low moringa concentration (T2, T4). Also, cadmium accumulation in gonads was decreased in all moringa-treated groups which may indicate the decrease of the toxic effect of cadmium on the reproductive system. In fish, most of the blood content is located in the internal organs, while muscles contain only 20% of the total blood volume, i.e. muscles are not very vascularized and such low blood supply weakens the chance of detoxicated materials to be stored and accumulated in fish muscles [73]. The present results are in agreement with Omotoso *et al*. [75] who mentioned that moringa possesses potent phytoactive components that can suppress nicotine toxicity in Wistar rats. Also, moringa seeds could protect Swiss mice from arsenic side effects and decreased arsenic concentration in soft tissues; liver (65%), kidney (54%), and blood (55%) [76]. Moreover, other medicinal plants can mitigate the toxic effects and reduce the accumulation of heavy metals in tissues. From them, fenugreek seed extract stimulated metallothionine up-regulation and decreased cadmium residues in the liver, gills, and muscles of Nile tilapia [47]. Also, curcumin reduced iron residues in serum, muscle, gonads, and kidney of catfish [48]. Cinnamon powder reduced lead accumulation in the whole body of Nile tilapia [77], and onion extract reduced cadmium accumulation in gonads, liver, and muscles of Nile tilapia [49].

Conclusion

The current study highlighted the protective role of moringa (*M. oleifera*) leaves, both in their crude form and as an extract, in mitigating the toxic impact of cadmium on Nile tilapia (*O. niloticus*). The research revealed that cadmium exposure has retarded the growth and immunity of fish while increasing oxidative stress. Conversely, feeding on crude moringa leaves promoted growth performance (especially WG and SGR), enhanced the immune response, and reduced the toxic impact of cadmium on antioxidant enzymes without a negative influence on the physiological functions. Additionally, the dietary moringa decreased cadmium accumulation in fish muscles and promoted its detoxification and excretion via the liver. The most effective protective effect of moringa was observed with a 0.5% concentration of the crude form. Further investigation into the effects of active compounds extracted from moringa on the overall health of Nile tilapia could be beneficial. *Conflicts of interest*

The authors declare that they have no conflict of interest or financial or otherwise.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The study was approved by the Institutional Animal Care and Use Committee, National Research Centre, Egypt.

TABLE 1. Growth performance of Nile tilapia subjected to Cd toxicity while fed on crude and extract of moringa leaves

Parameters	C	Cd	T1	T2	T3	T4
WG (g)	1.91±0.30^{a}	1.38±0.40^{a}	1.98±0.42^{a}	1.71±0.12^{a}	-6.08±0.83^{b}	-4.18±0.90^{b}
SGR	0.19±0.03^{a}	0.16±0.04^{a}	0.22±0.05^{a}	0.16±0.01^{a}	-0.63±0.10^{b}	-0.51±0.12^{b}
HSI	2.26±0.05^{a}	1.37±0.03^{c}	1.86±0.08^{ab}	2.26±0.03^{a}	1.45±0.06^{bc}	1.63±0.19^{bc}
GSI	1.38±0.03^{a}	1.19±0.03^{a}	0.72±0.01^{a}	1.95±0.01^{a}	0.61±0.19^{a}	2.27±0.73^{a}
CF	1.48±0.03^{ab}	1.56±0.01^{a}	1.22±0.08^{b}	1.37±0.07^{ab}	1.55±0.10^{a}	1.23±0.08^{b}

The data are shown as the mean ± standard error.
Values with the same letter within the same row do not differ significantly

TABLE 2. Hematological parameters of Nile tilapia subjected to Cd toxicity while fed on crude and extract of moringa leaves

Parameters	C	Cd	T1	T2	T3	T4
RBC (x10^{6}/µl)	1.40±0.03^{a}	1.41±0.02^{a}	1.19±0.09^{a}	0.99±0.13^{a}	1.09±0.07^{a}	1.06±0.14^{a}
Hb (g/dl)	4.80±0.12^{a}	4.60±0.06^{a}	4.13±0.18^{ab}	3.95±0.20^{ab}	3.85±0.20^{ab}	3.70±0.45^{b}
HCT %	21.75±0.48^{a}	21.50±0.29^{a}	20.00±0.41^{a}	15.00±1.73^{b}	16.50±0.87^{b}	15.00±2.52^{b}
WBC (x10^{3}/µl)	5.30±0.15^{cd}	3.43±0.24^{e}	3.73±0.10^{de}	7.94±0.43^{a}	5.73±0.58^{bc}	7.33±0.81^{ab}
Lymphocytes(%)	63.00±3.12^{bc}	44.00±0.87^{d}	61.00±0.50^{c}	72.00±1.73^{a}	68.33±1.44^{ab}	58.33±1.44^{c}
Neutrophils (%)	29.50±0.50^{a}	39.00±0.58^{a}	32.75±0.48^{a}	20.00±2.31^{b}	21.00±4.04^{b}	31.50±1.19^{a}
Eosinophils(%)	3.50±0.48^{a}	5.50±0.29^{a}	3.50±0.65^{a}	4.00±0.01^{a}	4.50±0.29^{a}	5.70±1.35^{a}
Monocytes(%)	4.00±2.31^{a}	11.5±0.01^{a}	2.75±0.50^{a}	4.00±0.58^{a}	6.20±2.89^{a}	4.50±1.66^{a}

The data are shown as the mean ± standard error.
Values with the same letter within the same row do not differ significantly

TABLE 3. Immunological parameters of Nile tilapia subjected to Cd toxicity while fed on crude and extract of moringa leaves

Parameters	C	Cd	T1	T2	T3	T4
Total Protein (mg/ml)	27.13±1.86^{c}	36.38±2.28^{b}	40.63±2.54^{ab}	48.28±3.02^{a}	40.56±2.54^{ab}	42.12±2.64^{ab}
Albumin (mg/ml)	3.07±1.01^{c}	9.73±1.31^{ab}	6.25±1.22^{bc}	14.73±1.76^{a}	4.29±1.02^{bc}	4.02±1.02^{c}
Globulin (mg/ml)	24.06±0.87^{b}	26.66±1.00^{b}	34.38±1.32^{a}	33.55±1.41^{a}	36.27±1.56^{a}	38.10±1.67^{a}
Lysozyme activity (unit/ml)	444±3.12^{b}	435±3.61^{b}	450±3.81^{a}	455±2.43^{a}	319±2.56^{c}	370±2.71^{c}
Antiproteases(%)	83±2.56^{a}	82±3.78^{a}	77±5.32^{b}	78.5±4.34^{b}	66.8±6.45^{c}	69.3±4.38^{c}

The data are shown as the mean ± standard error.
Values with the same letter within the same row do not differ significantly

TABLE 4. Biochemical parameters of Nile tilapia subjected to Cd toxicity while fed on crude and extract of moringa leaves

Parameters	C	Cd	T1	T2	T3	T4
ALT (Unit/l)	34.88±0.12^{a}	38.13±0.73^{a}	33.15±1.28^{a}	35.22±0.28^{a}	37.28±1.40^{a}	36.36±2.08^{a}
AST (Unit/l)	127.42±1.12^{a}	102.33±5.75^{ab}	78.26±6.34^{b}	112.23±20.0^{ab}	113.85±10.8^{ab}	132.90±4.9^{a}
Uric acid (mg/l)	46.80±1.22^{a}	43.38±0.85^{a}	42.54±2.60^{a}	44.32±1.09^{a}	39.68±1.95^{a}	35.99±2.17^{a}
Creatinine (mg/dl)	0.77±0.03^{a}	1.12±0.01^{a}	1.49±0.12^{a}	1.34±0.32^{a}	0.92±0.03^{a}	0.77±0.03^{a}
ALP (Unit/l)	35.50±0.82^{c}	47.00±1.81^{a}	45.00±0.37^{ab}	44.90±0.95^{ab}	49.20±2.32^{a}	38.50±0.86^{bc}
Triglycerides (mg/dl)	184.8±1.12^{b}	210.0±3.00^{a}	200.0±5.42^{a}	204.0±2.02^{a}	245.0±4.73^{a}	204.0±1.78^{a}
Cholesterol (mg/l)	75.49±0.91^{e}	91.21±1.70^{d}	305.33±16.3^{b}	438.57±21.8^{a}	189.54±19.1^{c}	42.13±15.6^{e}
Glucose (mg/l)	298.4±12.2^{b}	329.4±14.8^{a}	359.5±17.5^{a}	308.56±10.5^{b}	342.94±28.4^{ab}	177.12±29.8^{c}

The data are shown as the mean ± standard error.
Values with the same letter within the same row do not differ significantly

TABLE 5. Hepatic antioxidants activities (unit/ml) of Nile tilapia subjected to Cd toxicity while fed on crude and extract of moringa leaves

Parameters	C	Cd	T1	T2	T3	T4
CAT	146.43±2.12^{cd}	197.40±4.25^{b}	125.31±4.93^{d}	179.57±8.32^{bc}	260.03±15.3^{a}	199.33±8.20^{b}
POD	0.30±0.01^{c}	0.33±0.05^{bc}	0.51±0.04^{abc}	0.77±0.14^{a}	0.54±0.11^{abc}	0.70±0.06^{ab}
SOD	6.71±0.06^{c}	7.33±1.01^{c}	5.23±0.61^{c}	10.63±0.64^{a}	10.22±0.47^{ab}	7.77±0.36^{bc}
GST	0.03±0.01^{c}	0.61±0.09^{a}	0.38±0.10^{ab}	0.08±0.02^{bc}	0.18±0.04^{bc}	0.33±0.09^{abc}

The data are shown as the mean ± standard error.
Values with the same letter within the same row do not differ significantly

TABLE 6. Cadmium residues (mg/kg wet weight) in different organs of Nile tilapia subjected to Cd toxicity while fed on crude and extract of moringa leaves

Parameters	C	Cd	T1	T2	T3	T4
Liver	4.83±8.2^{d}	43.72±4.25^{c}	100.72±5.1^{a}	93.90±7.3^{ab}	90.02±5.3^{b}	92.90±4.2^{b}
Gills	1.45±0.11^{c}	6.91±1.05^{b}	6.12±2.04^{b}	8.14±2.24^{a}	10.36±2.21^{a}	8.94±1.46^{a}
Gonads	3.52±0.06^{c}	7.04±1.71^{a}	2.81±1.61^{c}	5.42±0.64^{b}	3.60±0.97^{c}	3.44±0.36^{c}
Muscle	0.02±0.11^{c}	0.42±0.09^{a}	0.44±0.10^{a}	0.11±0.52^{b}	0.44±0.24^{a}	0.22±0.09^{b}

The data are shown as the mean ± standard error.
Values with the same letter within the same row do not differ significantly

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تأثير أوراق المورينجا على الحالة الصحية لأسماك البلطي النيلي المعرض لسمية الكادميوم

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الملخص

اكتسبت النباتات الطبية مؤخرًا المزيد من الاهتمام في الحفاظ على صحة الأسماك. تركز هذه الدراسة على استخدام أوراق المورينجا (الخام والمستخلص) كمضاف علفي لتعزيز صحة البلطي النيلي (*Oreochromis niloticus*) بما في ذلك النمو والمناعة والإنزيمات المضادة للأكسدة في ظل وجود سمية الكادميوم. قسمت الأسماك إلى ست مجموعات: مجموعتان ضابطتان (واحدة سلبية (C) وأخرى موجبة معرضة لكلوريد الكادميوم (Cd)). تم تعريض المجموعات الأربع الأخرى لكلوريد الكادميوم بينما تم تغذيتها بـ 1% (T1) و0.5% (T2) من أوراق المورينجا الخام و1% (T3) و0.5% (T4) من مستخلص أوراق المورينجا. بعد 30 يومًا، تم تقييم النمو والعوامل الدموية والكيميائية الحيوية والمناعية والإنزيمات المضادة للأكسدة وبقايا الكادميوم في الأنسجة المختلفة. أظهرت نتائج مجموعة Cd انخفاضا معنويا في زيادة الوزن (WG)، معدل النمو النوعي (SGR)، عدد خلايا الدم البيضاء (WBC)، نسبة الخلايا الليمفاوية، ونشاط الليزوزيم. كما أظهرت زيادة معنوية في مستويات البروتين والألبومين والفوسفاتيز القلوي والدهون الثلاثية والجلوكوز والكوليسترول والكاتلاز (CAT) ومستويات الجلوتاثيون-S-ترانسفيراز (GST). كشفت مجموعات المورينجا الخام عن تحسن كبير في نشاط WBC والخلايا الليمفاوية والبروتين والألبومين والجلوبيولين والليزوزيم وانخفاض في قيم CAT وGST مقارنة بمجموعة Cd. كما سجلوا زيادة في WG وSGR مقارنة بمجموعة Cd. أظهرت الدراسة الحالية أن المورينجا تؤدي إلى تراكم الكادميوم في الكبد والخياشيم، إلا أنها كانت أقل بشكل ملحوظ في الغدد التناسلية والعضلات في المجموعات المعالجة بالمورينجا. وخلصت الدراسة إلى أن أوراق المورينجا الخام، وخاصة بتركيزات منخفضة (0.5٪)، آمنة كإضافات علفية لتعزيز الصحة العامة والنمو والمناعة في البلطي النيلي، والتخفيف من الآثار السامة للكادميوم.

الكلمات الدالة: أوراق المورينجا، البلطي النيلي، مؤشرات النمو، الحالة المناعية، مضادات الأكسدة، بقايا الكادميوم.