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Research Article

Ameliorative Effects of *Salvia hispanica* Seeds Against Zinc Oxide Nanoparticles Toxicity Inducing Hemato-Biochemical Variables, Immune-Oxidative Stress, and DNA Damage in *Oreochromis niloticus*

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The current study is a new approach for investigating the ameliorative effects of chia seeds powder (CSP) (*Salvia hispanica*) against haemato-biochemical dysfunction, oxidative stress, and DNA damage induced by zinc oxide nanoparticles (ZnONPs) in *Oreochromis niloticus*. Four fish groups were allocated in triplicates as follows: group I-control, group II-treated with 10 g/kg diet CSP, group III-exposed to 1/20 LC_{50} of ZnONPs, and group IV-exposed to 1/20 LC_{50} of ZnONPs + 10 g CSP/kg diet for 30 days. Blood, liver, and kidney tissue samples were collected at the end of the experiment for haemato-biochemical, oxidative stress biomarkers, and DNA damage evaluations. Results revealed that CSP administration significantly ameliorated the ZnONPs toxic effect. Where, CSP effectively increased WBCs, and serum total proteins, albumin, globulin, and immune parameters (IgG, IGM, and lysozyme [LYZ] activity), which were decreased after exposure to ZnONPs toxicity. In addition to the prominent ability of CSP to decrease the elevated levels of stress indicators (glucose and cortisol), liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), kidney products (creatinine and urea), catalase (CAT), superoxide dismutase (SOD), and hepato–renal lipid peroxidation (LPO) of Nile tilapia exposed to ZnONPs. Furthermore, CSP sustained the activity of the reduced glutathione (GSH), total antioxidant capacity (TAC), and WBCs, Hb, and Ht content were significantly declined in ZnONPs-exposed *O. niloticus* compared to the control group. Meanwhile, administration of 10 g CSP/kg diet restored the hemato-biochemical profile and reduced oxidative damage induced by ZnONPs toxicity as the WBCs and hepatic DNA damage were significantly

increased in exposed fish. The study suggested that CSP treatment has hepato-renal protective functions and antioxidative effect against ZnONPs toxicity in Nile tilapia.

Keywords: biochemistry; chia seeds; DNA fragmentation; Nile tilapia; zinc oxide nanoparticles

1. Introduction

Aquaculture production has increased significantly to meet the global uprising demands [1]. Nile tilapia is a popular cultured freshwater fish in many countries including Egypt for its various positive properties for example rapid growth, adaption to a wide variety of culture systems, and profitability [2, 3].

Cultured fish species are exposed to various challenges among which are toxicity and pollutants. Among those toxicants challenging the aquaculture industry, come the nanotoxicats [4–6]. Nanotechnology has gained great importance recently due to their implementations in various safe applications as dietary or in-water supplementations in aquaculture [7, 8]. However, nanomaterials' toxicity is probable to occur to the aquatic ecosystem [4, 9, 10]. The zinc oxide nanoparticles (ZnONPs) absorption is greater than its traditional form, and then undergoes bioaccumulation in different fish tissues [11]. They can cause detrimental hematological effects, in addition to gills and liver damage in Nile tilapia that can find its way to reach the human consumer [12–14].

Herbal supplementations prove their positive effects especially in ameliorating the toxicants impacts in aquaculture [15–18]. Chia seeds are considered the palatable seeds of *Salvia hispanica* which is originated from Northern, Mexico, and Guatemala [19]. Chia seeds own a huge nutritional profile and potent health-boosting privileges [20]. Chia seed carries 39% oil, which composes of fatty acids [21]. In addition, it is rich in carbohydrates (26%–41%), fats (30%–33%), proteins (15%–25%), dietary fiber (18%–30%), minerals, vitamins, and antioxidants [22, 23]. The active ingredients for chia seeds powder (CSP) play a major role in boosting antioxidant activity, immune system, and cellular function [24, 25]. They also have anti-inflammatory activity, beside to reducing blood glucose levels and blood pressure [26]. In aquaculture practices, Chia seeds dietary inclusion has been reported to enhance health performance and immune function [27]. In Nile tilapia, addition of chia seed powder to diet proves beneficial impact on growth efficacy, blood picture, and immune response [28], besides, it augments antioxidant capacity [29]. Additionally, chia seeds are utilized either total or partial replacement of fish oil in the diet of *Sparus aurata*, L. [30].

Few studies address the role of chia seed in ameliorating the toxicity with nanoparticles. Therefore, the current study was designed to assess toxic effects of ZnONPs on the haemato-biochemical, oxidative stress, and DNA fragmentation parameters and the possible role of CSP in ameliorating that toxicity in *O. niloticus*.

2. Materials and Methods

2.1. Ethical Statement. All experimental procedures were established by the Animal Welfare and Ethical Review Committee of Faculty of Veterinary Medicine, Sadat City University, Egypt (VUSC-014-1-24). All experimental procedures were conducted in line with ARRIVE guidelines and following the National Institutes of Health for Use and Treatment of Laboratory Animals.

2.2. Chemicals and Diet Preparation. Zinc oxide was obtained from Lab grade—Sigma Company. Dried *Salvia hispanica* seeds were obtained from Mashreq Company for Business Development in Smouha area (Alexandria), Egypt. Supplements Salvia seeds powder was added to a control diet (30% crude protein [CP]) at levels of 0% (control) and 1% (Table 1). The diets ingredients including salvia were carefully mixed with 100 mL of water per 1 kg of diet to make dough. The dough was then grinded using a meat grinder, and the resultant strings were kept to dry for a day prior to be crushed to get 1-mm diameter pellets. The diets were stored at 4°C for further usage. The ingredients were used to produce the diets including fishmeal (8.5%) and soybean meal (46.5%).

Chemical kits for biochemical assessment of urea, glucose, creatinine, cortisol, albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and total antioxidant capacity (TAC) were bought from Biodiagnostic Company, Dokki, Cairo, Egypt. Serum IgM and IgG levels were determined by the protocol of ELISA kits (Cusabio Biotech Co. Ldt, USA). ZnONPs were synthesized and the LC_{50} of ZnONPs was chosen according to Goda et al. [31]. Concentration of CSP (10 g/kg diet) used in dietary was chosen according to Abd El -Naby et al. [32].

2.3. Animal Acclimation and Monitoring Procedures for the Experiment. A total number of 200 healthy Nile tilapia, *Oreochromis niloticus* were collected from Al-Qanatir Al-Khayria, El-Qalyubia Governorate, Egypt and arrived in plastic bags. The average body weight of fish was 20.00 ± 2.50 g and the average length was 7 ± 2.40 cm. Fish were kept in 60 L glass aquaria measuring (30 cm × 80 cm × 40 cm) containing dechlorinated tap water for 2 weeks for acclimatization. The exchange of water was carried out at rate of 20% in each aquarium twice weekly and all the wastes were siphoned every day. Fish received a basal pellet diet (3% of body weight per day). Laboratory circumstances were maintained at 2000 L/cm conductivity; 7.3 ± 0.3 pH; 88%–95% oxygen saturation; 27 ± 2°C water temperature; 150 mg/L total hardness as CaCO3; 12:12 light/dark photoperiod.

2.3.1. Experimental Design. For the commencement of the experiment, four groups ($n = 50$ per group) in triplicates of fish were divided randomly as follows: group I (control), group II was exposed to CSP (10 g/kg diet), group Ill was exposed to 1/20 LC_{50} of (ZnONPs), and group IV was exposed to 1/20 LC_{50} of (ZnONPs) + CSP (10 g/kg diet) for 30 days. Blood,

TABLE 1: Ingredients and proximate analysis (%; on dry matter basis) of diets containing *Salvia hispanica*.

Ingredients	*Salvia hispanica* level (%)	
	0.0 (Control)	1.0
Fish meal (72% crude protein)	8.5	8.5
Soybean meal (45% crude protein)	46.5	46.5
Wheat bran	18.3	18.3
Ground corn	10.0	10.0
Corn oil	2.0	2.0
Cod liver oil	2.0	2.0
Mineral mixture[a]	3.0	3.0
Vitamin mixture[b]	3.0	3.0
Starch	6.7	6.2
Salvia hispanica powder	0	10
Total	100	100
Chemical composition (%)		
Dry matter	91.5	91.3
Crude protein	30.7	30.3
Total lipids	7.1	6.9
Crude fiber	4.8	4.9
Total ash	6.1	5.9

[a]Vitamin premix (per kg of premix): thiamin, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; paraaminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.
[b]Mineral premix (g/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$, 127.5; KCl,50.0; NaCl, 60.0; $FeC_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $Cu(OAc)_2{\cdot}H_2O$, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.03.

liver, and kidney tissue samples were collected at the end of experiment for the analysis of haemato-biochemical parameters and oxidative stress biomarkers.

2.4. Sampling. At the end of the experiment, seven fish from each treatment were collected and exposed to anesthesia with 0.02% benzocaine solution in the Laboratory. Samples of blood were collected from the caudal vessels and left to clot at room temperature in dry clean centrifuge tubes prior being centrifuged at 800 × *g* for 10 min at 4°C to permit immunological and biochemical assessments.

2.4.1. Biochemical Analysis. All biochemical assessments were carried out using commercial kits (Bio-Diagnostic Co., Cairo, Egypt). Levels of serum cortisol and glucose were determined using the protocols mentioned by Foster and Dunn [33]. Serum ALT and AST were determined by Reitman and FrankeSerum [34]. Albumin and total protein were assessed using the assay defined by Doumas et al. [35] and Henry [36]. Globulin was computed in serum by subtracting the value of albumin from the total protein value in the same sample. Urea and creatinine were measured by Coulombe and Favreau [37] and Larsen [38].

2.4.2. Assessment of Antioxidant Enzymatic Activity and LPO in Tissue. Tissue samples from kidneys and hepatopancreas were exposed to homogenization in phosphate buffer saline (0.2 M, pH 7.5) using Teflon homogenizer and a Potter–Elvejhem glass. The homogenate product was subjected to centrifugation at 1600 × *g* at 5°C for 15 min. The supernatant was kept at −20°C until further analysis. SOD activity was assessed using the assay defined by Nishikimi et al. [39]. CAT was evaluated depending on the protocol of Aebi [40]. Level of LPO were analyzed based on Uchiyama and Mihara [41]. Levels of reduced GSH and TAC were determined using the protocols of Beutler [42] and Koracevic et al. [43] using commercial kit from Gamma Trade Co., Cairo, Egypt.

2.4.3. Immunological Assessments. Lysozyme (LYZ) level was monitored in serum using a turbidimetric protocol using *Micrococcus luteus* in phosphate buffer (pH = 6.2) as a target [44]. Immunoglobulin (IgM) was calculated based on Secombes [45] protocol, The IgM level was triggered in polyethylene glycol, the preliminary and last total proteins were subtracted to get the value of IgM.

2.4.4. Measurement of DNA Fragmentation in Liver. The fragmentation of DNA for liver specimens of tilapia fish was evaluated based on the protocol mentioned by Kurita-Ochiai et al. [46] using a spectrophotometer (Micro lab 200 Vital Scientific Dieren, The Netherlands) at 575 or 600 nm against reagent blank. The percentage of fragmented DNA was measured as follows:

$$\text{Percentage of fragmented DNA} = \text{Fragmented DNA}/(\text{fragmented DNA} + \text{intact DNA}) \times 100.$$

2.5. Statistical Analysis. The findings were existing as means ± standard error. Analysis of data were done statistically using analysis of variance, one-way ANOVA, to assess impacts of ZnONPs toxicity and dietary chia seeds. Variances among means were verified at the 5% probability level using Duncan's multicomparison test. All the statistical analyses procedures were performed using SPSS (version 20; SPSS, Richmond, VA, USA).

3. Results

3.1. Haemato-Biochemical Profile. Biochemical parameters including hepatic enzymes (ALT and AST), glucose and cortisol, and renal indicators (creatinine and urea) were significantly ($p < 0.05$) elevated in group III after exposure to 1/20 LC_{50} ZnONPs. Group IV showed that CSP was effective in decreasing elevated levels of ALT, AST, glucose, cortisol, creatinine, and urea in addition to returning them to a level close to that of the group I. Cortisol and creatinine readings showed the most significant decrease for the group IV in comparison to other groups. Total proteins, albumin, and globulin were decreased in response to ZnONPs exposure in group III and 10 g CSP/kg was effective in elevating their levels close to groups I and II levels (Table 2).

All the hematological parameters including counts of RBCs and WBCs, Hb readings showed nonsignificant ($p < 0.05$)

Table 2: Blood biochemical parameters (means ± SE) in Nile tilapia, *Oreochromis niloticus*, exposed to 1/2 LC_{50} ZnONPs and co-administrated with (10 g/kg diet), respectively, for 30 days.

Parameters	Groups			
	Group I	Group II	Group III	Group IV
AST (μ/L)	45.13 ± 0.42^{b}	45.17 ± 0.34^{b}	59.24 ± 0.41^{a}	46.19 ± 0.10^{b}
ALT (μ/L)	19.04 ± 0.17^{b}	18.02 ± 0.24^{b}	30.24 ± 0.08^{a}	17.65 ± 0.07^{b}
Glucose (mg/dL)	84.42 ± 0.14^{b}	86.37 ± 0.21^{b}	115.51 ± 0.51^{a}	89.02 ± 0.24^{b}
Cortisol (μg/dL)	15.58 ± 0.16^{b}	14.27 ± 0.10^{c}	23.51 ± 0.11^{a}	14.51 ± 0.20^{c}
Total proteins (g/dL)	7.83 ± 0.32^{a}	7.95 ± 0.27^{a}	3.25 ± 0.16^{c}	6.85 ± 0.30^{b}
Albumin (g/dL)	3.24 ± 0.31^{a}	3.30 ± 0.22^{a}	1.31 ± 0.26^{b}	3.76 ± 0.15^{a}
Globulin (g/dL)	4.59 ± 0.31^{a}	4.65 ± 0.28^{a}	1.94 ± 0.21^{c}	3.09 ± 0.10^{b}
Creatinine (mg/dL)	0.57 ± 0.02^{b}	0.56 ± 0.03^{b}	0.94 ± 0.01^{a}	0.51 ± 0.02^{c}
Urea (mg/dL)	13.60 ± 0.17^{b}	12.84 ± 0.13^{c}	18.61 ± 0.04^{a}	13.01 ± 0.1^{b}

Note: Group I, fish served as control group and fed on free basal diet. Group II, fish served as control group + (CSP) (10 g/kg diet). Group III, fish exposed to 1/20 LC_{50} ZnONPs for 30 days. Group IV, fish exposed to 1/20 LC_{50} ZnONPs + (CSP) (10 g/kg diet) for 30 days. Means with different superscript lowercase letters in the same row for each parameter are significantly different ($p < 0.05$).

Table 3: Hematological parameters (mean ± SE) in Nile tilapia, *Oreochromis niloticus*, exposed to 1/20 LC_{50} ZnONPs and co-administrated with (CSP) (10 g/kg diet), respectively, for 30 days.

Groups	Parameters			
	RBCs (10^6/mm^3)	WBCs (10^6/mm^3)	Hb (g/dL)	Ht (%)
Group I	4.60 ± 0.02^{a}	0.68 ± 0.11^{a}	8.42 ± 0.37^{a}	32.25 ± 1.05^{a}
Group II	4.48 ± 0.05^{a}	0.69 ± 0.12^{a}	8.34 ± 0.46^{a}	33.30 ± 1.06^{a}
Group III	3.29 ± 0.13^{c}	0.94 ± 0.31^{c}	4.71 ± 0.19^{c}	22.15 ± 1.24^{b}
Group IV	4.04 ± 0.10^{b}	0.61 ± 0.05^{b}	8.02 ± 0.25^{b}	33.20 ± 1.17^{a}

Note: Group I, fish served as control group and fed on free basal diet. Group II, fish served as control group + (CSP) (10 g/kg diet.). Group III, fish exposed to1/20 LC_{50} ZnONPs for 30days. Group IV, fish exposed to 1/20 LC_{50} ZnONPs + (CSP) (10 g/kg diet) for 30 days. Means with different superscript lowercase letters in the same column for each parameter are significantly different ($p < 0.05$).

differences among group II and group I. However, group III revealed that the exposure to 1/20 LC_{50} ZnONPs caused significant ($p < 0.05$) decrease in RBCs, WBCs counts, and Hb reading compared to the other groups. Supplementation by 10 g CSP/kg diet was effective in significantly ($p < 0.05$) rising this level as manifested in group IV compared to the rest of groups (Table 3).

3.2. Antioxidants. Hepatic and renal antioxidants showed the same response pattern to LC_{50} ZnONPs exposure. Hepatic and renal antioxidants including LPO, SOD, and CAT showed significant ($p < 0.05$) elevation in group III in response to 1/20 LC_{50} ZnONPs exposure. Supplementation with 10 g CSP in group IV demonstrated significant ($p < 0.05$) decrease in this elevated level and returning to the control group I. However, group II did not show a significant difference before exposure to ZnONPs related to group I (Figures 1 and 2).

Also, for levels of hepatic and renal GSH and TAC readings, no significant ($p < 0.05$) differences were detected among different groups. Except in group III after exposure to ZnONPs that significantly ($p < 0.05$) decreased GSH and TAC levels in comparison to groups I, II, and IV. Group IV showed significant ($p < 0.05$) amelioration through rising levels of GSH and TAC in comparison to group III and returning to the control level as in group I (Figures 1 and 2).

3.3. Immune Response. Activities of IgG, IgM, and LYZ did not reveal significant ($p < 0.05$) differences except in group III after exposure to ZnONPs that significantly ($p < 0.05$) decreased their levels. Group IV showed significant ($p < 0.05$) elevation in IgG, IgM, and LYZ activities in comparison to group III and returned them to the control level as in group I and group II before exposure to ZnONPs (Figure 3).

3.4. DNA Fragmentation. Liver DNA fragmentation showed a significant ($p < 0.05$) increase after exposure to ZnONPs in group III, and group IV showed marked ($p < 0.05$) decrease to this effect in comparison to ZnONPs exposed group III. In addition, group II before exposure to ZnONPs showed a significant decrease compared to the control group I (Figure 4).

4. Discussion

Toxicity with nanoparticles in aquaculture is regarded as a major concern. In this respect, herbal therapies have been growing and denote a major implement for establishing a sustainable aquaculture industry and relieving toxicity impacts [5, 6, 14, 47, 48]. Here in, we assessed the toxic effects of ZnONPs on the haemato-biochemical, antioxidant activity, and DNA damage indicators and the ameliorative antitoxic effects of CSP against ZnONPs in *O. niloticus*.

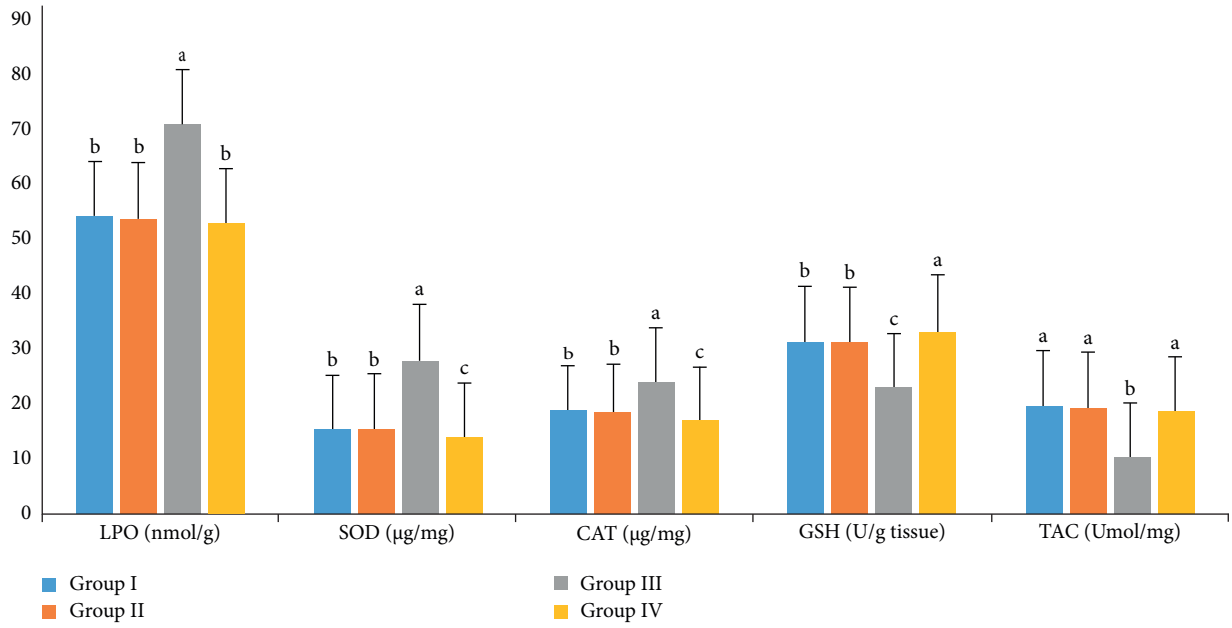

FIGURE 1: Effect of 1/20 LC_{50} of ZnONPs exposure and dietary CSP (10 g/kg diet) on antioxidant profile in kidney homogenate of Nile tilapia, *Oreochromis niloticus* for 30 days. Means with different lowercase letters in the same column for each parameter are significantly different ($p < 0.05$). Group I: fish served as control group and fed on free basal diet. Group II: fish served as control group + (CSP) (10 g/kg diet). Group III: fish exposed to 1/20 LC_{50} ZnONPs for 30 days. Group IV: fish exposed to 1/20 LC_{50} ZnONPs + (CSP) (10 g/kg diet) for 30 days.

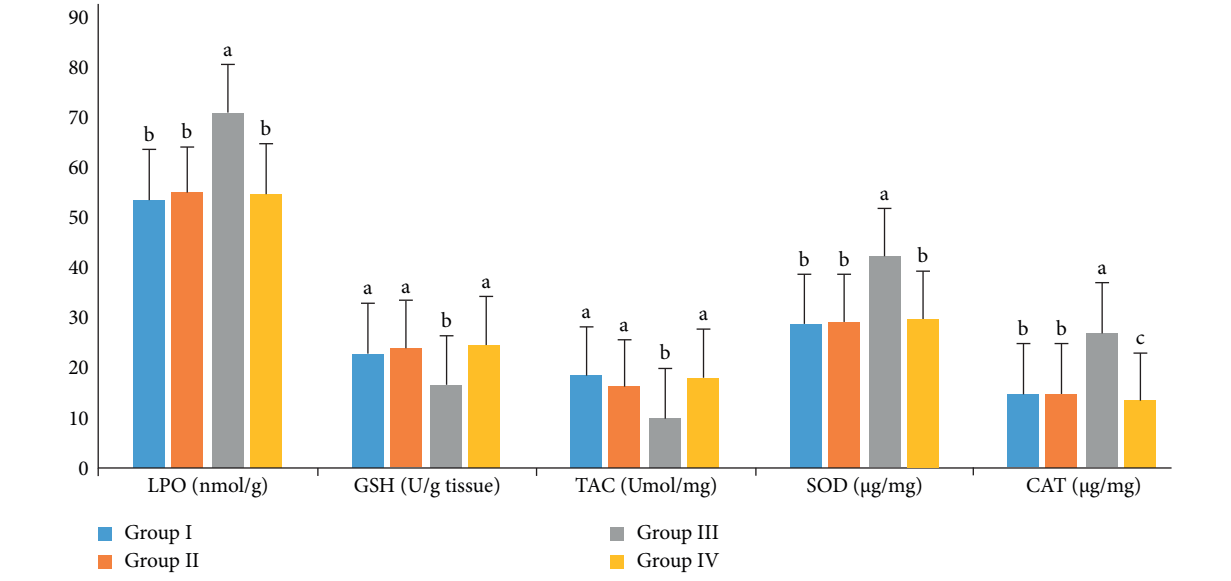

FIGURE 2: Effect of 1/20 LC_{50} of ZnONPs exposure and dietary CSP (10 g/kg diet) on antioxidant profile in liver homogenate of Nile tilapia, *Oreochromis niloticus*, for 30 days. Means with different lowercase letters in the same column for each parameter are significantly different ($p < 0.05$). Group I: fish served as control group and fed on free basal diet. Group II: fish served as control group + (CSP) (10 g/kg diet). Group III: fish exposed to 1/20 LC_{50} ZnONPs for 30 days. Group IV: fish exposed to 1/20 LC_{50} ZnONPs + (CSP) (10 g/kg diet) for 30 days.

Biochemical measurements are essential in monitoring the status of fishes [49]. The present study implied to occurrence of disturbance in blood glucose and hepato-renal function. Similarly, Kaya et al. [50] monitored imbalance in blood serum glucose upon exposure of tilapia fish to ZnONPs. Furthermore, Sherif et al. [51] mentioned an elevation in hepatic enzymes in Nile tilapia induced by zinc oxide toxicity. Additionally, the current findings were supported by an in vivo study of Liu et al. [52] who elucidated the mechanism of ZnONPs toxicity on kidney function via inducing apoptosis in tubular epithelial cell and elevated serum creatinine and urea nitrogen. Interestingly, the CSP administration was effective in ameliorating the ZnONPs harmful effects via regenerating the biochemical parameters including hepatic enzymes, glucose, cortisol, and

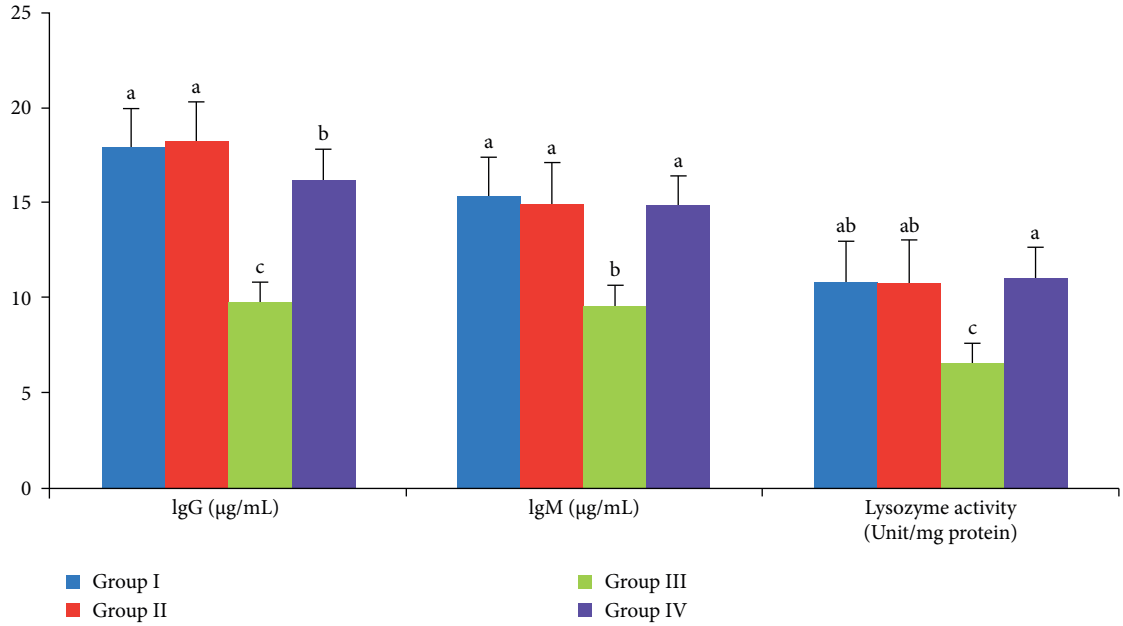

FIGURE 3: The innate immune parameters (means ± SE) in Nile tilapia, *Oreochromis niloticus*, exposed to 1/20 LC_{50} ZnONPs and co-administrated with (CSP) (10 g/kg diet), respectively, for 30 days. Means with different lowercase letters in the same column for each parameter are significantly different ($p < 0.05$). Group I: fish served as control group and fed on free basal diet. Group II: fish served as control group + (CSP) (10 g/kg diet). Group III: fish exposed to 1/20 LC_{50} ZnONPs for 30 days. Group IV: fish exposed to 1/20 LC_{50} ZnONPs + (CSP) (10 g/kg diet) for 30 days.

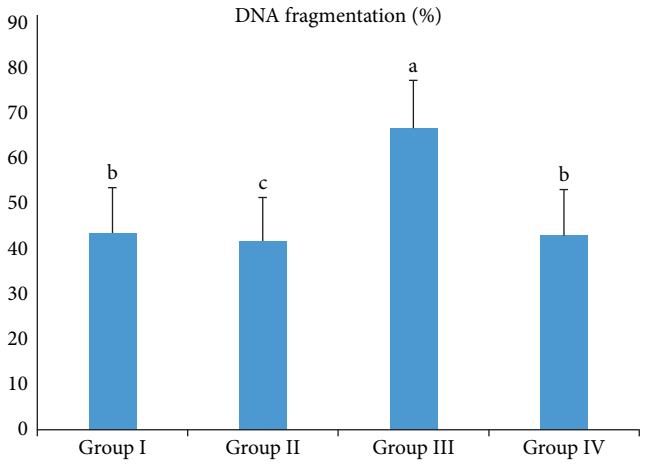

FIGURE 4: Liver DNA fragmentation (mean ± SE) in Nile tilapia, *Oreochromis niloticus*, exposed to 1/20 LC_{50} ZnONPs and co-administrated with (10 g/kg diet) for 30 days. Means with different lowercase letters in the same column for each parameter are significantly different ($p < 0.05$). Group I: fish served as control group and fed on free basal diet. Group II: fish served as control group + (CSP) (10 g/kg diet). Group III: fish exposed to1/20 LC_{50} ZnONPs for 30 days. Group IV: fish exposed to 1/20 LC_{50} ZnONPs + (CSP) (10 g/kg diet) for 30 days.

renal indicators. On the same instance, Abd El-Naby et al. [32] found a marked increase in serum RBA, TP, ALB, and GLO levels were in CSP-fed Nile tilapia. In addition, Mahmoud et al. [28] found that CSP supplementation significantly improved ($p < 0.05$) serum lipids, glucose, and cortisol levels in Nile tilapia subjected to cold stress. These results could be attributed to the hepatoprotective and antioxidative roles of CSP through the action of its active ingredients as reported by Uribe-Martínez et al. [53].

Hematological profiles are crucial bioindicators for fish performance and toxicological conditions [54–56]. In the current study, we noted hematological changes after exposure to ZnONPs toxicity which was ameliorated by CSP supplementation. Similarly, Rasheed et al. [57] reported significant differences in the hemato-biochemical parameters of common carp in response to ZnONPs, reflecting their dangerous effects especially if reached their toxic levels. In addition, Hamed et al. [13] also reported hematological alterations in Nile tilapia in response to ZnONPs toxicity. Focusing on the significance of CSP, the ameliorative activity of CSP was proved previously by Ofori-Mensah et al. [30] which includes enhancing the antioxidant system and preventing hemolysis via its active compounds ingredients (phenolic, phytosterols, carotenoids, and flavonoids compounds) [53]. These compounds have the ability to protect red blood cells from hemolysis and possess antioxidant properties as described by Mudgil et al. [58] and Rabail et al. [59]. Similarly, Abd El-Naby et al. [32] and Mahmoud et al. [28] reported a positive relationship between WBCs, RBCs, Hb, and Ht values with CSP concentrations in diets. Also, Elabd et al. [7] and Rashidian et al. [60] found significantly improved hemato-biochemical picture of Nile tilapia received nano iron oxide-supplemented diets; and nutmeg extract of common carp (*Cyprinus carpio*).

Hepatic and renal antioxidants are a successful indicator to fish's response and health conditions [61]. Our findings

showed that hepatic and renal antioxidants including LPO, SOD, and CAT showed significant ($p < 0.05$) increases after ZnONPs exposure and supplementation with 10 g CSP significantly ($p < 0.05$) decreased this elevated level and returned to the control. Likewise, Sherif et al. [62] verified an increase in the antioxidant biomarkers including hepatic glutathione peroxidase (GPx) and CAT, upon exposure of Nile tilapia to ZnONPs stress. Moreover, Abdelazim et al. [63] found elevation in reduced GSH levels, SOD, reduced GSH, and CAT following exposure of Nile tilapia to ZnONPs. On the same instance, Abd El-Naby et al. [32] and Mahmoud et al. [28] confirmed the enhancing properties of CSP to antioxidative activities of GPx, CAT, and SOD in Nile tilapia fingerlings. The ameliorating activities are related to the prominent antioxidative action of chia seed and its ability to initiate free radicals scavenging and protecting lipids from peroxidation. This is owed to the CSP active compounds (phenolic, tocopherols, carotenoids, flavonoids, and phytosterols) as clarified by Ahmadifar et al. [64] and Rahim et al. [65].

The remarked antioxidative properties of CSP supplemented diets are mostly translated in improved immune response parameters, such as LYZ, and IgM and IgG immunoglobulins activities [7, 61]. Our findings revealed that ZnONPs exposure decreased the levels of IgG, IgM, and LYZ activities and CSP supplemented group showed significant ($p < 0.05$) amelioration in these levels and returning them to control. Similarly, Hamed, Amen, et al. [13] and Rasheed et al. [57] reported hazardous impacts of exposure to ZnONPs on the immune-related parameters. Concurrent with Abd El-Naby et al. [32] and Mahmoud et al. [28], CSP supplementation is recorded to improve LYZ, and IgM and IgG in Nile tilapia.

Immune and antioxidants-related parameters are good biomarkers for DNA and cellular damages, which is related to free radicals production and impairment in their scavenging [66]. Current findings showed that exposure to ZnONPs increased the levels of DNA fragmentation and CSP was effective in decreasing those elevated levels. In a line with an in vitro study conducted by Kukla et al. [67], he noticed that the DNA damage was elevated with increasing concentration of ZnONPs. Another report by Attia et al. [68] noted that fragmentation of DNA was augmented upon ZnONPs exposure which verified by increased percentage of tailed DNA. On the other hand, CSP administration demonstrated positive results in lessening DNA damage owing to the immunostimulant, antioxidant, and cellular protective activity as described by Mahmoud et al. [28], Rabail et al. [59], and Rahim et al. [65].

5. Conclusions

The role of chia seed in ameliorating the toxicity was highlighted in the current study. Where ZnONPs exposure deteriorated the hematological, biochemical, immune, and antioxidative stress parameters. Chia seeds were effective in amending the toxic effects of ZnONPs via enhancing hemato-biochemical, hepato-renal function, oxidative stress, and DNA fragmentation parameters in *O. niloticus*. Future studies are required to figure the action of feed incorporations on mitigating the toxicity.

Data Availability Statement

All the data are available in this manuscript.

Ethics Statement

All the experimental procedures were established by the Animal Welfare and Ethical Review Committee of Faculty of Veterinary Medicine, Sadat City University, Egypt (VUSC-014-1-24). All the experimental procedures were conducted in line with ARRIVE guidelines and following the National Institutes of Health for Use and Treatment of Laboratory Animals.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

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