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RESEARCH

Dietary deacetylated chitin nanoparticles confer protection against diazinon toxicity in male African catfish: evaluation of immune-biochemical, antioxidant, and reproductive profiles

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Abstract Currently, deacetylated chitin (chitosan) nanoparticles (CNPs) are successfully utilized in aquaculture practices. This trial demonstrates the efficacy of CNPs in combating diazinon (DZN) toxicity in African catfish, *Clarias gariepinus*, via monitoring hepato-renal function, serum immune trait, hormonal function, and hepato-renal antioxidant activity. Four groups were allocated as follows: a control group, a CNPs group (0.66 ml/L CNPs), a DZN exposed group (0.598 ppm, 1/10 LC50), and a DZN + CNPs group (0.598 ppm DZN + 0.66 ml/L CNPs), all for 30 days. Exposure to 0.598 PPm DZN resulted in a severe decline in the immune parameters (albumin, globulin, immunoglobulins (IgG, IgM), and total proteins), neurological indicator, acetylcholinesterase (AchE), reproductive hormones (Testosterone (T.) and Luteinizing Hormone (LH)), and the superoxide dismutase (SOD) and total antioxidant capacity (TAC) readings in both hepatic and renal samples. Moreover, a clear increment in hepatic and renal indicators

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(AST, ALT, urea, and creatinine), lipid peroxidation (LPO), and some reproductive indices including follicle stimulating hormone (FSH) and serum 17-β estradiol (E2) was clearly increased. Interestingly, the dietary inclusion of CNPs markedly palliated the toxicity by DZN with significant improvement in the immune-reproductive indices, plus normalizing the values of hepato-renal function and augmenting the activity of antioxidant parameters**.** Thus, the present study demonstrates the efficacy of CNPs in mitigating low-dose DZN toxicity, resulting in significant improvements in physiological, biochemical, and reproductive parameters. This highlights the promising potential of CNPs as a viable strategy for enhancing the health of *C. gariepinus*, thereby promoting the sustainability of the aquaculture industry and safeguarding human health.

Keywords Chitosan nanoparticles · Diazinon toxicity · Male *Clarias gariepinus* · Immune-biochemical · Reproductive hormones

Introduction

Aquatic animals are exposed to pesticide pollution that could get absorbed primarily via the gills and constitutes a health hazard when the polluted fish enters the human food chain (Vali et al. 2022). Diazinon (DZN) is one of the organophosphate pesticides that can reach aquatic ecosystems through unintentional spills, drifts, leakage, overspills, and agricultural applications (Stara et al. 2020). It has an appraised mild to high toxicity to freshwater, brackish, and saltwater fishes (Flynn et al. 2020). Harmful effects include various critical hazards and devastating mortalities to non-target species and aquaculture ecosystem in correlation with the uprising of pesticides usage in direct or indirect ways (Soliman et al. 2023; Vali et al. 2022).

Diazinon is a toxic insecticide that procures its toxic effect through its capability to hinder the nervous systems of fish via affecting acetylcholinesterase (AChE) activity, and thus, disturbs the hormone release (Majhi et al. 2023). Diazinon in aquatic animals also impairs muscle control reducing physical strength, and causing spine deformities in larvae and juveniles (Sayed et al. 2023).

African Catfish (*Clarias gariepinus*) could bio-indicate the occurrence of toxicity (Soliman et al. 2019). That is because, its particular eating habits, exposure to various pollutants, and living habitat in sediment aggravate bio-accumulation process (Khalil et al. 2017).

Assessment of various immune response and stress bio-indicators (hematological, biochemical, and physiological) in response to nanoparticles (NPs) may deliver a precise view of the health status, indicating their overall performance (Abdel Rahman et al. 2022; Kakakhel et al. 2022; Mahboub et al. 2024). Chitosan NPs are cationic biopolymers that are reported to be active and nontoxic (El-Naggar et al. 2021). They are reported to boost growth performance, enhance immune-antioxidant traits, and defeat microbial infection in farmed fish (Abdel-Tawwab et al. 2019; Yu et al. 2023). It has been reported that CNPs are efficient in the removal of trace metals and chitosan nanogel has a verified role against heavy metal toxicity (Seyedmohammadi et al. 2016). Chitosan nanoparticles have the ability to enhance drug permeability, thus improving active pharmaceutical ingredients and favoring the entrance of chitosan nanoparticles in many applications. In addition, they own antioxidant activity through scavenging free radicals (Elabd et al. 2023; Zoe et al. 2023).

Here in, the objective of the present experimental trial is to assess various biomarkers (immune-biochemical, reproductive trait, and antioxidant enzymes) to investigate the detrimental effects of DZN exposure on African catfish with emphasis on the ameliorating effect of chitosan nanoparticles (CNPs) dietary incorporation.

Material and methods

Chemicals

Diazinon (DZN) was procured in the form of transparent-brownish liquid (Ciba – Geigycorp, Greensboro, NC27419-8300). Kits for evaluating the biochemical indicators (alanine aminotransferase (ALT), aspartate aminotransferase (AST), cortisol, albumin, total protein, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), lipid peroxidation

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(LPO), and total antioxidant capacity (TAC) were procured from the Egyptian Bio-Diagnostic company (Cairo, Egypt). In addition, kits for Ach E, Testosterone, Estradiol, FSH, and LH were acquired from the Egyptian Gamma Trade company (Cairo, Egypt).

Chitosan nanoparticles (CSNPs) preparation

Chitosan with low-molecular weight and 93% deacetylation was dissolved in 1 L acetic acid distilled water and left for 30 min on a magnetic stirrer. Followed by an addition of about 200 ml sodium tripolyphosphate at room temperature in drop by drop manner, then magnetic stirring to the chitosan solution was performed for 45 min to acquire the chitosan nanoparticles. The CSNPs can be kept at 2–8 °C until additional analysis, following Zx et al. (2007). The 0.66 ml/L CNPs dose was selected according to Hamed et al. (2021).

Fish acclimation

A total of 350 healthy male catfish, *C. gariepinus* (300 ± 5 g average weights, 42 ± 5 cm average length) were used in this study. Fish were transported from Abbassa Farm, Sharqia Government in large plastic containers supplied with battery aerators as an oxygen source. In the laboratory, fish were acclimatized in the 80 L glass aquaria for 2 weeks before the experiments. Fish received daily a commercial diet at a rate of 3% body weight, water temperature was 26 °C ± 1 °C, pH was 7.2 ± 0.2, dissolved oxygen recorded 6.4 ± 0.3 mg/L, alkalinity monitored 121 mg/L, and hardness was 151 mg/L calcium carbonate.

Experimental layout

The DZN half-lethal concentration (LC50) was selected according to Bakhshwan et al. (2009) as 5.98 ppm, and 1/10 LC50 of (DZN) was chosen for this study. Semi-static system was used in this experiment, at which the test solution was renewed after every 48 h. For the sublethal exposure, after 2 weeks of acclimation, four fish groups (each in triplicates) were allocated as: control (group I), group treated with 0.66 ml/L CSNPs (group II), group exposed to 0.598 PPm (1/10 LC50 of DZN) (group III), and group exposed to 0.598 PPm DZN + 0.66 ml/L CSNPs (group IV) for 30 days.

Blood sampling

After 30-day exposure time, six fish from each group were anaesthetized with benzocaine (0.02%). Blood was obtained from the fish caudal vessels, left to clot at room temperature, then centrifuged for 15 min at 5000 × g and 4 °C to obtain sera for the assessment of the biochemical parameters.

Investigation of biochemical parameters

The activities of serum aspartate aminotransferase (AST, catalog No.; EK12276, Biotrend Co., Maryland, USA) and alanine aminotransferase (ALT, catalog No.; MBS038444, MyBioSource Co., California, USA) in serum were detected following Reitman and Frankel (1957). Cortisol was assayed following active® cortisol RIA kit; serum albumin (Catalog No.; MAK125) and total protein (Catalog No.; MBS9917835) were estimated following the protocols reported by Palladino et al. (2019) and Doumas et al. (1971). Globulin level in serum was estimated by deducting the level of albumin from the total value of protein in the same sample. The brain Ach E activity (Catalog No.; MAK119; Sigma-Aldrich) was determined spectrophotometrically following the protocol of Knedel and Böttger (1967). All estimated biomarkers were performed using a spectrophotometer (Lambda EZ201; Perkin Elm, Beaconsfield, UK).

Reproductive hormones examination

The levels of LH and FSH in serum samples were ELISA measured following the protocols of (Jt and P 1998). Serum levels of both and17-β Estradiol E2 and testosterone T were ELISA measured based on (Bell 1995).

Immune response parameters

Nitroblue tetrazolium dye was used to evaluate the whole blood sample respiratory burst (RB) activity following Abdel-Tawwab et al. (2018) and Solem et al. (1995). Polyethylene glycol precipitation was used to measure total immunoglobulin (IgM) through subtraction of initial and final total protein following procedures of Anderson et al. (1995).

Estimation of antioxidant and lipid peroxidation activities

Hepatic and renal tissue samples were homogenized in cold (0.1 M pH 7.4) phosphate buffer using a Teflon homogenizer. After filtration (1600 rpm, 4 °C, and 10 min), centrifugation was performed to the homogenate; resulting supernatant was stored at − 20 °C. The level of superoxide dismutase (SOD) was estimated according to the method illustrated by Nishikimi et al. (1972). The reduced glutathione (GSH) levels were detected using the method of Aebi (1984). LPO levels were monitored following Mihara and Uchiyama (1978). Total antioxidant capacity (TAC) was estimated according to the method of Solem et al. (1995). Catalase (CAT) level was measured following Aebi (1984).

Statistical analyses

Analysis of variance, one-way "ANOVA" (version 20; SPSS, Richmond, VA, USA), and Duncan's multiple range tests were used to statistically analyze obtained data with two factors of the effects of CPF toxicity and the dietary PE. Means differences were estimated at a probability level of 5% following Liu (2015).

Results

Biochemical assays

Liver enzymes (AST and ALT) and cortisol levels were significantly ($P < 0.05$) increased after exposure to 1/10 LC_{50} diazinon, and 0.66 ml/L CNPs effectively ameliorated this elevation (Table 1). Kidney biomarker enzymes (urea and creatinine) were also significantly ($P < 0.05$) elevated after 1/10 LC_{50} diazinon exposure, and a significant ($P < 0.05$) reduction was recoded in response to chitosan nanoparticles (CNPs) (0.66 ml/L) incorporation (Table 1).

However, levels of AchE were markedly decreased after exposure to 1/10 LC_{50} diazinon, and CNPs incorporated diet significantly ($P < 0.05$) ameliorated this affect by increasing AchE and returning to the control level before toxicity exposure. In addition, proteins (albumin, globulin, and total proteins) were significantly ($P < 0.05$) decreased in response to diazinon toxicity, and the incorporated group (0.66 ml/L CNPs) significantly ($P < 0.05$) elevated those levels and returned them to levels quite similar to the control group before diazinon exposure (Table 1).

Table 1 Biochemical parameters (means ± SE) in male African catfish, *C. gariepinus* exposed to 1/10 LC_{50} diazinon (DZN) and co-administrated with chitosan nanoparticles (CNPs) (0.66 ml/L), respectively for 30 days

Groups Parameters	1st group	2nd group	3rd group	4th group
µ/LAST	40.05 ± 0.51^{c}	41.01 ± 0.43^{c}	62.07 ± 0.57^{a}	48.70 ± 0.39^{b}
ALT µ/L	18.23 ± 0.07^{b}	18.71 ± 0.14^{b}	25.11 ± 0.02^{a}	16.01 ± 0.18^{c}
Cortisol mg/dl	11.27 ± 0.12^{bc}	11.60 ± 0.34^{bc}	18.91 ± 0.06^{a}	12.60 ± 0.34^{b}
Urea mg/dl	15.90 ± 0.34^{b}	15.71 ± 0.36^{b}	23.10 ± 0.03^{a}	15.42 ± 0.16^{bc}
Creatinine mg/dl	0.69 ± 0.03^{b}	0.67 ± 0.05^{bc}	0.94 ± 0.01^{a}	0.70 ± 0.04^{b}
AchE µ/L	422.03 ± 1.07^{a}	420.97 ± 1.15^{a}	369.01 ± 2.60^{b}	424.21 ± 1.12^{a}
Total proteins g/dl	7.43 ± 0.04^{a}	7.18 ± 0.03^{a}	3.28 ± 0.16^{c}	6.95 ± 0.07^{b}
Albumin g/dl	2.46 ± 0.19^{a}	2.50 ± 0.16^{a}	1.41 ± 0.06^{c}	2.39 ± 0.05^{b}
Globulin g/dl	4.97 ± 0.14^{b}	4.58 ± 0.16^{a}	$\pm 0.04^{c}1.87$	4.56 ± 0.20^{a}

Means with different superscript letters in the same row for each parameter are significantly different ($P < 0.05$)

1st group: Male catfish served as control group and fed on free basal diet

2nd group: Male catfish served as control group + (CNPs) (0.66 ml/L)

3rd group: Male catfish exposed to 1/10 LC_{50} diazinon for 30 days

4th group: Male catfish exposed to 1/10 LC_{50} diazinon + (CNPs) (0.66 ml/L) for 30 days

AST aspartate transaminase, *ALT* alanine aminotransferase, *AchE* acetylcholinesterase

Reproductive profile

Before exposure to DZN, reproductive hormones did not reveal any significant ($P<0.05$) difference than the control group. While, after diazinon exposure reproductive hormones (LH and T.) were significantly ($P<0.05$) decreased, and chitosan nanoparticles (0.66 ml/L CNPs) supplementation significantly ($P<0.05$) ameliorated this action and elevated those levels (Table 2).

However, FSH, and 17-β E2 hormones were significantly ($P<0.05$) increased after diazinon toxicity exposure, and the nano chitosan diet (0.66 ml/L CNPs) significantly ($P<0.05$) reduced those levels (Table 2).

Hepatic and renal antioxidant profile

Although, all renal antioxidants (LPO, CAT, SOD, GSH, and TAC) did not reveal any significant difference before exposure to diazinon toxicity, LPO, CAT, and SOD were significantly ($P<0.05$) increased after exposure to diazinon toxicity, and chitosan nanoparticles significantly ($P<0.05$) decreased those elevated levels. However, GSH and TAC levels were significantly ($P<0.05$) decreased after exposure to diazinon toxicity, and chitosan nanoparticle diets significantly ($P<0.05$) elevated their levels (Tables 3 and 4).

Similarly, hepatic antioxidants (LPO, CAT, SOD, GSH, and TAC) showed a significant difference only after exposure to diazinon toxicity. Exposure to diazinon toxicity significantly ($P<0.05$) increased LPO, CAT, and SOD activities, and chitosan nanoparticles significantly ($P<0.05$) decreased those elevated levels. However, diazinon toxicity significantly ($P<0.05$) decreased GSH and TAC levels, and chitosan nanoparticles diets significantly ($P<0.05$) elevated their levels (Tables 3 and 4).

Discussion

Aqua feed supplementation with various natural plant treatments is being widely used recently for various purposes involving growth enhancer, immune stimulation, and ameliorating oxidative stress status (Elabd et al. 2022; Hamed et al. 2022; Rashidian et al. 2022). Nevertheless, the enhancing actions of chitosan nanoparticles to compete the diazinon toxicity and improve the male *C. gariepinus* fertility are inadequate (Elabd et al. 2023; Goda et al. 2023; Hamed et al. 2022; Mahboub et al. 2022; Mohamed et al. 2020). The negative effects of organophosphate pesticides (OPs) including diazinon on aquatic ecosystems appear to be due to the inappropriate handling and distribution (Barathinivas et al. 2022; Díaz-Resendiz et al. 2019; Mahi et al. 2022; Ravi et al. 2023). The current study revealed that diazinon negatively affected biochemical, antioxidant, and reproductive profiles of male catfish, and those CNPs significantly mitigated this effect positively.

Regarding the biochemical indicators, which are considered important indicators for toxicity with OPs that cause reduction of enzymatic activities in fishes

Table 2 Reproductive hormones (mean ± SE) in male African catfish, *C. gariepinus* exposed to 1/10 LC_{50} diazinon (DZN) and co-administrated with chitosan nanoparticles (CNPs) (0.66 ml/L), respectively for 30 days

Parameters Groups	LH (μ/L)	FSH (μ/L)	T. (g/ml)	17-β E2 (g/ml)
1st group	0.81 ± 0.05^{a}	0.41 ± 0.02^{b}	76.53 ± 0.42^{a}	237.61 ± 0.35^{b}
2nd group	0.82 ± 0.07^{a}	0.42 ± 0.05^{b}	76.47 ± 0.31^{a}	238.42 ± 0.25^{b}
3rd group	0.54 ± 0.02^{c}	0.55 ± 0.03^{a}	50.02 ± 0.07^{c}	338.01 ± 0.40^{a}
4th group	0.79 ± 0.09^{b}	0.38 ± 0.02^{c}	70.99 ± 0.92^{b}	241.16 ± 0.13^{c}

Means with different superscript letters in the same row for each parameter are significantly different ($P<0.05$)

1st group: Male catfish served as control group and fed on free basal diet

2nd group: Male catfish served as control group + (CNPs) (0.66 ml/L)

3rd group: Male catfish exposed to 1/10 LC_{50} diazinon for 30 days

4th group: Male catfish exposed to 1/10 LC_{50} diazinon + (CNPs) (0.66 ml/L) for 30 days

LH luteinizing hormone, *FSH* follicle stimulating hormone, *T.* testosterone, *17-β E2* 17-β estradiol

Table 3 Effect of 1/10 LC_{50} of diazinon (DZN) exposure and CNPs (0.66 ml/L) on antioxidant profile in kidney tissues of male African catfish, *C. gariepinus* for 30 days

Items	LPO (nmol/g)	SOD (Ug/mg)	CAT (Ug/mg)	GSH (U/g tissue)	TAC (Umol/mg)
1st group	60.52 ± 2.53^{b}	27.26 ± 2.14^{bc}	16.31 ± 0.93^{c}	36.05 ± 1.06^{a}	18.31 ± 0.16^{a}
2nd group	60.40 ± 2.41^{b}	27.34 ± 2.11^{bc}	16.01 ± 0.47^{c}	37.09 ± 1.14^{a}	17.35 ± 0.14^{a}
3rd group	81.26 ± 2.36^{a}	36.01 ± 0.92^{a}	30.04 ± 0.23^{a}	20.02 ± 0.09^{c}	8.91 ± 0.32^{c}
4th group	65.10 ± 2.35^{c}	29.05 ± 1.49^{b}	20.35 ± 0.11^{b}	33.32 ± 1.22^{b}	14.90 ± 0.18^{b}

Means with different superscript letters in the same row for each parameter are significantly different ($P < 0.05$)

1st group: Male catfish served as control group and fed on free basal diet

2nd group: Male catfish served as control group + (CNPs) (0.66 ml/L)

3rd group: Male catfish exposed to 1/10 LC_{50} diazinon for 30 days

4th group: Male catfish exposed to 1/10 LC_{50} diazinon + (CNPs) (0.66 ml/L) for 30 days

LPO lipid peroxidation, *SOD* superoxide dismutase, *CAT* catalase, *GSH* reduced glutathione, *TAC* total antioxidant capacity

Table 4 Effect of 1/10 LC_{50} of diazinon (DZN) exposure and CNPs (0.66 ml/L) on antioxidant profile in liver tissues of male African catfish, *C. gariepinus* for 30 days

Items	LPO (nmol/g)	GSH (U/g tissue)	TAC (Umol/mg)	SOD (Ug/mg)	CAT (Ug/mg)
1st group	59.09 ± 1.52^{c}	36.61 ± 1.30^{a}	20.35 ± 2.01^{a}	33.49 ± 2.04^{b}	16.31 ± 0.25^{c}
2nd group	57.05 ± 1.30^{c}	35.32 ± 1.24^{a}	20.48 ± 2.04^{a}	31.93 ± 2.08^{b}	16.97 ± 0.38^{c}
3rd group	74.03 ± 2.53^{a}	25.19 ± 0.52^{c}	9.97 ± 1.23^{c}	42.04 ± 0.21^{a}	30.71 ± 0.19^{a}
4th group	62.10 ± 2.44^{b}	34.05 ± 1.42^{ab}	16.38 ± 2.49^{b}	30.00 ± 2.50^{b}	20.03 ± 0.46^{b}

Means with different superscript letters in the same row for each parameter are significantly different ($P < 0.05$)

1st group: Male catfish served as control group and fed on free basal diet

2nd group: Male catfish served as control group + (CNPs) (0.66 ml/L)

3rd group: Male catfish exposed to 1/10 LC_{50} diazinon for 30 days

4th group: Male catfish exposed to 1/10 LC_{50} diazinon + (CNPs) (0.66 ml/L) for 30 days

LPO lipid peroxidation, *GSH* reduced glutathione, *TAC* total antioxidant capacity, *SOD* superoxide dismutase, *CAT* catalase

(Chinnadurai et al. 2022; Glusczak et al. 2007), CNPs significantly ($P < 0.05$) reduced AST, ALT, urea, and creatinine, and cortisol that were elevated in response to 1/10 LC_{50} diazinon exposure. Similarly, AST and ALT were significantly ($P < 0.05$) increased in *C. gariepinus* exposed to diazinon (Al-Otaibi et al. 2019) and to polyvinyl chloride micro-particles (Iheanacho and Odo 2020). Moreover, diazinon exposed rainbow trout (*Oncorhynchus mykiss*) showed increased liver enzymes activities (Banaee et al. 2023; Shabanzadeh et al. 2023). Those elevated levels are suggested to be because of the damage to liver that could lead to liberate large quantities of these enzymes into the blood and thus could be a sensitive indicator of cellular damage (Al-Asgah et al. 2015; Al-Otaibi et al. 2019). The ameliorating properties of the chitosan could be attributed to their antioxidant properties and decreasing the histological changes in liver tissue (Thilagar and Samuthirapandian 2020). Thus, it is assumed a promoting favorable rule of CNPs on mitigating hepatic damage induced by diazinon toxicity.

Insecticides are known to decrease the acetylcholinesterase activity, causing decreased motility in the fish (Vali et al. 2022). In the present study, AchE was significantly decreased in response to diazinon toxicity, and the CNPs significantly normalized those activities. In the same pattern, Zebrafish (*Danio rerio*) exposed to different doses of deltamethrin showed a suppression of acetylcholinesterase (Petrovici et al. 2020). That is because of the inhibitory effect of the

organophosphate pesticide (diazinon) on the activity of acetylcholinesterase, responsible for dissociation of acetylcholine, a neurotransmitter included in nerve signaling (Souza et al. 2023). Toxicity usually causes hepatic damage that is manifested with a decrease in total protein levels (Iheanacho et al. 2021; Iheanacho and Odo 2020).

In the present study, AchE, albumin, globulin, and total proteins were significantly decreased in response to diazinon toxicity, and the CNPs significantly normalized those activities. Herein, exposure to diazinon significantly decreased total protein, albumin, and globulin levels in rainbow trout, and silymarin supplementation significantly ameliorated those actions and returned readings to the normal levels (Banaee et al. 2023). Those decreased levels are because diazinon toxicity may affect the liver's ability to produce or maintain normal proteins levels resulting in reduced levels in the bloodstream. These alterations could adversely influence the overall well-being, imbalance in fluid transport, and suppressed immune response (Rashidian et al. 2020). Similarly, *C. gariepinus* exposed to polyvinyl chloride micro-particles showed a remarked decrease in total protein levels (Iheanacho and Odo 2020). The CNPs normalizing activities are because of their potential to improve the liver's ability to produce essential proteins such as globulins and albumin by decreasing the oxidative stress challenges and destruction in the liver (Chien et al. 2016; Ngo and Kim 2014). The current findings also revealed elevated urea and creatinine levels which are waste products produced from muscle metabolism and released via blood through the kidneys indicating kidney dysfunction (Banaee et al. 2023). The mitigating properties of CNPs are because they can ameliorate the oxidative stress conditions and act as a safeguard toward the integrity of kidneys stopping waste products accumulations in the bloodstream (Abdelkhalek et al. 2017; Chien et al. 2016; Elabd et al. 2023).

The reproductive hormones could be used as biomarkers for gonadotoxicity (Petrovici et al. 2020). Current findings showed that reproductive hormones LH and T. significantly decreased, while FSH, and 17-β E2 hormones were significantly increased after diazinon toxicity and the nano chitosan diets significantly ameliorated those levels. Those effects are due to the endocrine disruption properties of diazinon, through impairing the production, discharge, and transport of reproduction hormones and reproduction regulators, thus causing metabolic disorders and oxidative stress (Darvishi et al. 2022). Similarly, diazinon reduced 17β-Estradiol in serum of *Lepomis macrochirus* (Bugel et al. 2011; Darvishi et al. 2022). The antagonizing properties of chitosan could be attributed to its ability to sustain fish growth, immune response, and antioxidation (Thilagar and Samuthirapandian 2020).

Diazinon may cause oxidative damage through reducing the metabolic pathways, involving different tissues mitochondrial electron permeability and redox enzymes (Shabanzadeh et al. 2023). In the present study, diazinon exposure significantly elevated hepatic and renal LPO, and TAC activities, plus hepatic SOD, but diminished GSH and TAC levels. On the same context, challenging the rainbow trout with diazinon caused severe damage in kidneys and liver with depression with alterations in the antioxidants activities, which were ameliorated with commercial astaxanthin (Shabanzadeh et al. 2023). Same findings were reported with Banaee et al. (2023) and Ming et al. (2020) for alterations in the antioxidant status in response to diazinon toxicity. In addition, superoxide dismutase, catalase, and glutathione peroxidase activities were found to decrease markedly in *C. gariepinus* exposed to polyvinyl chloride micro-particles (Iheanacho and Odo 2020). Chitosan was able to sustain the antioxidative status caused by ROS and also to prevent damages to liver and kidneys (Elabd et al. 2023; Thilagar and Samuthirapandian 2020).

Conclusion

The present study's findings showed that dietary CNP supplementation was effective in mitigating low-dose DZN toxicity. This was manifested through the significant improvements in physiological, biochemical, and reproductive parameters. This indicates the potential of CNPs on enhancing the health of *C. gariepinus*, thereby promoting the sustainability of the aquaculture industry.

Author contribution Conceptualization: H.E., H.H.M., H.S.H., A.A.A, E.M.Y., A.R., S.H.O., S.M.S., S.J.D., Z.H. Methodology: H.E., H.H.M., H.S.H., A.A.A, S.K., E.M.Y., A.R., S.H.O., S.M.S., S.J.D., Z.H. Software and data curation: H.E., H.H.M., H.S.H., A.A.A, E.M.Y., S.K., A.R., S.H.O., S.M.S., S.J.D., Z.H. Writing-Original draft preparation: H.E., H.S.H., H.H.M., Writing- Reviewing and Editing: H.H.M., H.E.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Institutional review board statement All experimental procedures with live fish were agreed by the animal welfare and ethical review committee of the Faculty of Veterinary Medicine, Sadat City University, Egypt (VUSC-035–1-23). All experimental procedures were performed in compliance with the ethical guidelines approved by the National Institutes of Health for Use and Treatment of Laboratory Animals.

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