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Immune-protective, antioxidant and relative genes expression impacts of β-glucan against fipronil toxicity in Nile tilapia, *Oreochromis niloticus*

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ABSTRACT

Our study is considered to attempt reducing the immune-toxic and antioxidant impacts of exposure to fipronil (FP) on Nile tilapia, *Oreochromis niloticus* using the β-glucan (βG). Two hundred and seventy fingerlings of Nile tilapia were divided randomly into six groups (45 tilapias of each, in 3 replicates): group I control (CT) group nourished on a basal diet. Group II (βG) nourished a basal diet supplemented with 0.4% βG. Groups III (1/20 FP) and V (1/10 FP) was exposed to 1/20 and 1/10 of the 96 h LC_{50} of FP in water and nourished the basal diet respectively. Groups IV (1/20 FP + βG) and VI (1/10 FP + βG) were exposed to 1/20 and 1/10 FP concomitantly with 0.4% βG supplementation for 90 successive days. Growth performance metrics were higher in βG group than CT. While those metrics were fallen at exposure to 1/20 or 1/10 FP. Supplementation with βG elevated the IgM and lysozyme levels.Whereas, tilapias exposed to FP only at different concentration showed lowering of those compared to CT. Supplementation with βG was effectively augmented IgM and lysozyme in 1/20 FP exposed tilapias. Furthermore, in a minor grade at 1/10 FP exposed tilapias. Exposure to FP increased the activities of hepatic markers chiefly at 1/10, however the βG supplementation was successfully improved these markers. There was imbalance of cortisol level at FP exposure where, βG combining to FP alleviate this disparity. There was fallen in LDH, MDH and FDPase in βG tilapias where continuing raise in 1/10 FP followed by 1/20 FP. βG supplementation raise the level of GSH, without significant variations in MDA conversely occurs in FP alone. Genes expression of βG caused raise of both GPx and GR, without fluctuations in CAT and SOD. Exposure to FP diminishes all evaluated antioxidant genes. It could fulfilled that supplementation with βG successfully alleviated the immune-toxic and antioxidant impact of FP in tilapias.

1. Introduction

Freshwater ecosystems face the most pollution worldwide [1]. They are subjected to many stressors, including weather changes, acidic substances, sewage pollution and chemical substances, which are the most common pollution sources in freshwater [2]. The random use of chemicals, especially pesticides in agriculture presents a serious risk to all aquatic organisms, including fish by polluting the ecosystem [3,4]. Some itemized that remainders of chemical pollutants are higher in freshwater ecosystems than in other ecosystems [5].

Fipronil (FP) is one of the supreme broadly used global insecticides for rice field protection. FP is a phenylpyrazole broad-spectrum insecticide, which depleted as a main insecticide in some countries on their market because other commonly products as lindane, dieldrin, and DDT have been associated with many drawbacks and their use has been outlawed. Low FP concentrations have a powerful results on insects and unique pests [6]. Bioaccumulation of FP in fish, causing genotoxic, cytotoxic impacts and even mortality [7]. In addition, FP metabolites, as sulfide and sulfone are extra toxic than FP itself [8,9]. Many recent readings have tested the toxicity of FP in aquatic ecosystem varying among 0.001–10.004 μg/L [10,11].

Immunostimulants are natural or synthetic substances that can be used to improve immunity in fish and fish protection against infection by increasing the production of antibodies [12,13]. Natural immunostimulants are widely used on fish farms to improve fish immunity [14]. β-glucan (βG) is a widely used immunostimulant that can be

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obtained from bacteria, plants, yeast, algae and mushrooms. βG chemically considered as a group of heterogeneous polysaccharides with different structures and forms producing immune effects by enhancing lysozyme and complement system activity, thus improving the bactericidal and phagocytic effects of fish phagocytes and increasing fish resistance to bacteria [12,15].βG use in the diets of fish greatly convalesces fish immunity [16,17]. βG amend stress reply anti variant stressors either infection or ecological [18–22].For model, βG use as a diet supplement has been allied with increase specific and nonspecific immunity in diversity of fish species including yellow croaker [23],Asian catfish [24], Zebrafish [25].

Recently, most researchers resorted to the use of natural elements as immunostimulants for bettering of immunity or hinder of serious disorders [26,27], especially using of βG for augment the immune response [14]. Given the encouraging findings from the previously published investigates using βG as an applicable immunostimulant, this study considered to attempt reducing the immune-toxic and antioxidant impacts of exposure to FP on *O.niloticus* using the βG.

2. Material and methods

2.1. Chemicals

2.1.1. Fipronil

Fipronil ($C_{12}H_4C_{l2}F_6N_4OS$) (99.1% pure) was purchased from (Bio Quest International Private Limited, Mumbai, India). The 96 h LC_{50} of FP for *O. niloticus* is 0.042 mg/l [28].

2.1.2. β-glucan

A commercially available βG (Best Choice Pharma in Sharkia Province, Egypt), added by 0.4% to the basal diet [29].

2.2. Fish management and maintenance

A total of 270 fingerlings of Nile tilapia were purchased from the Abbassa Fish Hatchery in Sharkia Province, Egypt with an average with an average (34 ± 0.5 g weight and 11 ± 0.05 cm length). The fish were divided randomly in eighteen glass aquaria (96 L, 15 tilapias / each) and allowed to acclimate to the new laboratory surroundings for 10 days before the arising of the experiment. During the experiment, fish were maintained in dechlorinated tap water under continual aeration and the following water parameters were conquered in our concern: pH 6.4 ± 0.2, non-ionized ammonia 0.8 ± 0.01 μg/l temperature 25.5 ± 2.0 °C, dissolved oxygen 5.1 ± 2.0 mg/l, and nitrite 0.06 ± 0.01 mg/l. The study was ratified by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University.

2.3. Design of the experiment

The fish were divided into six groups randomly (45 fish of each, in 3 replicates): group I (CT), control group, fed only a basal diet (formulation of the basal diet is described in Table 1). Group II (βG) was fed 0.4% βG supplemented diet. Group III (1/20 FP) was exposed to 0.0021 mg/l of the 96 h LC_{50} of FP in water and fed the basal diet. Group IV (1/20 FP + βG) was exposed to 0.0021 mg/l FP in water and fed the basal diet supplemented with 0.4% βG; group V (1/10 FP) was exposed to 0.0042 mg/l of the 96 h LC_{50} of FP in water and fed the basal diet. Group VI (1/10 FP+ βG) was exposed to 0.0042 mg/l the 96 h LC_{50} of FP in water and fed the basal diet supplemented with 0.4% βG, then blended mechanically and lastly machine pelleted. The direness of pellets at room temperature for 48 h and kept at 4 °C until use.

All fish were fed 5% Bwt. three times daily of their particular diets for 90 days. Conferring to the standard practices of all foodstuffs used in diet formulation were analyzed for dry matter (DM), crude protein (CP), and ether extract (EE) and crude fiber (CF). The diets were organized at the Fish Research Center, Faculty of Veterinary Medicine, Zagazig University, Egypt. The basal diet included 2944.41 kcal/kg metabolizable energy (ME) and 30.80% CP in the form of dry pellets and was created to meet the nutrient supplies of *O. niloticus* [30] (Table 1).

Table 1
Ingredients and calculated composition of the basal diet.

Ingredient %	
35	Yellow corn
10	Wheat flour
18	Soybean meal
16	Fish meal
14	Poultry byproduct meal
5.5	Vegetable oil
1.5	Vitamin and mineral mixture[a]
Calculated composition%	
84.28	Dry matter
30.79	Crude protein
9.92	Ether extract
2.40	Crude fiber
7.09	Ash
38.99	Nitrogen-free extract
2944.41	Digestible energy[b]

[a] Vitamin and mineral mixture (alfakema): each 1 kg contained 580000 IU vit. A, 8600 IU vit. D3, 720 mg vit. E., 142 mg vit. K3, 0.1 mg vit. C, 58 mg vit. B1, 34 mg vit. B2, 34 mg vit. B6, 58 mg vit. B12, 86 mg folic acid, 8 mg pantothenic acid, 65 mg manganese sulfate, 3000 mg zinc methionine, 2000 mg iron sulfate, 3400 mg copper sulfate, 572 mg cobalt sulfate, 25 mg sodium selenite, 25 mg calcium iodide, and calcium carbonate (carrier substance) to 1000 g.

[b] Digestible energy calculation based on values of 3.5 kcal/g protein, 8.1 kcal/g fat, and 2.5 kcal/g nitrogen-free extract (NFE).

2.4. Growth performance metrics

Weighting the fish were done at the beginning and the end of research. Feed conversion ratio (FCR), body weight gain % (BWG %) [31], also specific growth rate (SGR %) [32] were regulated.

2.5. Blood sampling

At the end of experiment, caudal blood vessels were used for drawn of blood samples in syringes without anticoagulant, then centrifugation at 202*g* for 15 min for serum formulation [33–36]. The serum were amassed and stored immediately at −20 °C until used for immunological and biochemical fortitude [26,37].

2.6. Serum immunological and biochemical investigation

Immunoglobulin M (IgM) level were determined [38] using an ELISA kit for fish with sensitivity 1.25 μg/ml (Catalog No. *CSB-E*12045Fh *(96k test)*; Cusabio Biotech Co., Ltd.). Lysozyme commotion was precise using a turbidity assay [38]. Alanine aminotransferase (ALT) activity was finalized using a Spectrum Kit, Egypt [39]. Serum cortisol level were finalized using a cortisol microtiter strip ELISA kit (DioMed. Cairo, Egypt) [40].

2.7. Tissue sampling for biochemical investigation and antioxidant activity determination

Liver tissues homogenates were assembled from all fish for fortitude of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), fructose-1,6-diphosphatase (FDPase), malondialdehyde (MDA) as lipid peroxidation marker and reduced glutathione (GSH) levels [41–45]. On the other hand, the brain homogenate acetylcholine esterase (AchE) activity appraisal [46]. The brain homogenate was obtained by (one g

Table 2
Primer sequences.

Gene name	Primer Sequence (5′→3′)	Gene accession number	Annealing temp °C.	Reference
SOD	F:5′ GGCTTTGATAAGGACAGTGGAAGACT 3′ R: 5′ GAAGTGGGACGAGACCTGTAGTG 3′	AJ492825	55	[56]
CAT	F: 5′ CGTCATATGAACGGATACGG 3′ R: 5′ TCAGCCTGCTCAAAGGTCAT 3′	GQ376154	55	[56]
GPx	F: 5′ CCAAGAGAACTGCAAGAACGA3′ R: 5′ CAGGACACGTCATTCCTACAC3′	EF206801	60	[55]
GR	F: 5′ CATTACCGAGACGCGGAGTT3′ R: 5′ CAGTTGGCTCAGGATCATTTG3′	EU887951	60	[55]
β-actin	F- 5′ CAATGAGAGGTTCCGTTGC3′ R- 5′ AGGATTCCATACCAAGGAAGG3′	DQ539421	55	[56]

SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; GR = glutathione reductase.

of brain tissue added to 10 mL sodium phosphate 0.1 M, 7.5 pH) homogenized in an ice bath homogenizer. The homogenate was centrifuged (30 min at 5.000 g in 5 °C), and the soluble part was castoff. The pellet was re-suspended (same volume of a 0.1 g % Triton X-100 in a 0.1 M sodium phosphate, 7.5 pH) and re-homogenized. A second centrifugation (90 min at 15.000 g in 5 °C), the subsequent supernatant was collected and frozen at −20 °C for AchE assay within 72 h [47].

2.8. Molecular determination

Total liver RNAs were extricated using a *Gene JET* RNA purification kit (Fermentas, UK) following the industrialist's decorum. The gotten total RNAs (hepatic CAT, SOD, GPx and GR) were gauged for quantity and quality by a Nano-Drop® ND-1000 Spectrophotometer (Wilmington, Delaware USA). cDNA was fashioned from pure samples by Superscript II RNase H- reverse transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) was applied in Bio-systems 2720 thermal cycler. cDNA (1 µl) was blended with 12.5 µl of 2^x SYBR® Green PCR Master Mix from BioRad, 1 µl (10 pmol/µl) of each primer and 9.5 µl of RNase and DNase free water (Sigma, UK). The primer sequences and circumstances were itemized in (Table 2).All primers were manmade by Sigma-Aldrich (Steinheim, Germany). β-actin gene was used as a controller gene for maintenance. The relative fold modifications were intended using the $2^{-\Delta\Delta Ct}$ method [48–50].

2.9. Statistical analysis

The gotten effects were analyzed by one-way ANOVA using SPSS-21 software [51]. Significant modifications among means were analyzed. Statistical significance defined as $P < 0.05$.

3. Results

3.1. Effect of βG and / or FP on growth performance metrics

The detailed records in (Table 3) reveal that, growth performance metrics such as final body weight (FBW), BWG % and SGR (%) were significantly higher in βG group than in the CT. While those metrics in the groups fed the basal diet with 1/20 or 1/10 FP correspondingly were significantly lower than those in CT. βG administration with FP improved FBW, BW gain %, especially in the group kept on 1/20 FP. The same trend in improvement was also noted for the total feed conversion ratio and food consumption.

3.2. Effect of βG and / or FP on immunological parameters

From the results illustrated in (Table 4), it is distinct that supplementation with βG significantly augmented the serum IgM and lysozyme levels when judged to the CT. Whereas, the groups subjected to 1/20 or 1/10 FP respectively in water without βG showed significantly lower of IgM and lysozyme levels when compared to CT. βG dietary supplementation with 1/20 FP was successfully amended IgM and lysozyme which not significantly diverse from levels in the CT. Additionally, but in a lesser degree, βG amended also IgM and lysozyme levels in 1/10 FP.

3.3. Effect of βG and / or FP on biochemical parameters

Exposure to FP, (Table 4) significantly intensified the serum of hepatic enzyme ALT activity remarkably at a dosage of 1/10 FP, whereas the βG dietary supplementation was profitably amended liver function as perceived by cutbacks in ALT level in varied dosage of FP. The group

Table 3
Effect of dietary supplementation with 0.4% βG on growth performance of *Oreochromis niloticus* exposed to 1/20 and 1/10 96-h LC_{50} FP.

Parameters	Groups					
	CT	βG	1/20 FP	1/20 FP + βG	1/10 FP	1/10 FP + βG
Initial BW (g)	34.00 ± 0.54	34.67 ± 0.89	34.00 ± 0.61	35.33 ± 0.67	34.67 ± 0.41	34.00 ± 0.53
Final BW (g)	54.00^{b} ± 0.78	60.69^{a} ± 0.68	47.00^{d} ± 0.81	51.00^{c} ± 0.55	40.00^{e} ± 0.37	46.00^{d} ± 0.62
BW gain (g)	20.00^{b} ± 0.15	26.02^{a} ± 0.12	13.00^{c} ± 0.21	15.67^{bc} ± 0.16	5.33^{d} ± 0.16	12.00^{c} ± 0.24
BW gain (%)	54.42^{b} ± 1.29	75.10^{e} ± 1.71	38.43^{c} ± 1.02	44.67^{bc} ± 0.91	15.43^{a} ± 1.71	35.43^{c} ± 1.00
Specific growth rate	0.48^{b} ± 0.03	0.62^{a} ± 0.14	0.36^{c} ± 0.51	0.41^{bc} ± 0.02	0.16^{d} ± 0.26	0.34^{c} ± 0.32
Feed consumption (g)	62.57^{ab} ± 0.19	63.73^{b} ± 0.25	64.77^{a} ± 0.23	64.11^{ab} ± 0.14	44.49^{c} ± 0.35	64.18^{ab} ± 0.15
Feed conversion ratio	3.12^{bc} ± 0.12	2.44^{d} ± 0.14	4.98^{b} ± 0.76	4.09^{bc} ± 0.18	8.34^{a} ± 0.82	5.34^{b} ± 0.56

Means in the same row with different superscripts are significantly different at $P < 0.05$.
CT = control, βG = , β-glucan BW = body weight, FP = fipronil.

Table 4
Effect of dietary supplementation with 0.4% βG on immune, biochemical and antioxidant parameters of *Oreochromis niloticus* exposed to 1/20 and 1/10 96-h LC_{50} FP.

Parameters	Groups CT	βG	1/20 FP	1/20 FP + βG	1/10 FP	1/10 FP + βG
IgM (μg/ml)	24.67^{b} ± 0.78	41.33^{a} ± 0.39	18.33^{c} ± 0.20	29.67^{b} ± 0.45	12.33^{e} ± 0.18	19.00^{c} ± 0.27
Lysozyme (μg/ml)	26.67^{b} ± 1.05	32.33^{a} ± 0.28	18.67^{c} ± 0.45	26.00^{b} ± 0.52	9.67^{d} ± 0.43	18.00^{c} ± 0.77
ALT (μg/ml)	16.33^{c} ± 0.73	14.00^{d} ± 0.81	30.67^{b} ± 0.24	18.00^{c} ± 0.23	67.33^{a} ± 1.21	31.33^{b} ± 0.33
Cortisol (μg/ml)	5.48^{c} ± 0.67	5.40^{c} ± 0.47	12.23^{b} ± 0.16	5.74^{c} ± 0.11	17.21^{a} ± 0.46	10.50^{b} ± 0.48
LDH (U/mg^{-1})	3.30^{b} ± 0.45	2.15^{c} ± 0.09	3.22^{b} ± 0.45	3.01^{bc} ± 0.10	4.28^{a} ± 0.37	3.34^{bc} ± 0.36
MDH (U/mg^{-1})	12.44^{c} ± 0.60	8.15^{d} ± 0.56	14.44^{b} ± 0.60	12.52^{c} ± 0.97	20.16^{a} ± 1.08	14.22^{b} ± 1.05
FDPase (g/mol^{-1})	3.36^{c} ± 0.15	2.25^{d} ± .26	4.49^{b} ± 0.12	3.88^{c} ± 0.35	6.29^{a} ± 0.27	4.91^{b} ± 0.41
AchE (μg/mg)	5.23^{a} ± 0.49	5.30^{a} ± 0.45	4.01^{b} ± 0.43	5.29^{a} ± 0.66	2.92^{d} ± 0.21	3.74^{c} ± 0.38
MDA (nmol/g)	14.35^{cd} ± 0.46	13.48^{d} ± 0.39	19.65^{b} ± 0.82	15.66^{c} ± 0.78	24.27^{a} ± 0.84	18.96^{b} ± 0.65
GSH (nmol/mg)	9.23^{ab} ± 0.23	11.62^{a} ± 0.25	6.18^{c} ± 0.84	8.92^{b} ± 0.76	4.21^{d} ± 0.45	6.44^{c} ± 0.48

Means in the same row with different superscripts are significantly different at $P < 0.05$.
CT = control, βG = , β-glucan, FP = fipronil, IgM = Immunoglobulin M, ALT = Alanine aminotransferase, LDH = lactate dehydrogenase, MDH = malate dehydrogenase, FDPase = fructose-1, 6-diphosphatase, AchE = acetylcholine esterase, MDA = malondialdehyde,GSH = reduced glutathione.

treated with βG only showed non-significant variances compared with the control group.

The hormonal level, non-significant variances were noted in cortisol level among CT, βG, and 1/20 FP + βG. However, 1/10 FP and 1/20 FP showed significantly intensify in the serum cortisol in correlate to CT. βG help decline the cortisol level when supplemented with 1/10 FP equated to 1/10 FP alone.

Concerning to, LDH, MDH and FDPase, βG group showed significant decrease in their activities when compared with other groups. The significant highest levels of LDH, MDH and FDPase were reported at 1/10 FP followed by 1/20 FP, while these activities were significantly ameliorated by feeding on a diet supplemented with βG chiefly and subjected to 1/20 FP.

In a different way AchE levels were significantly drop in the groups exposed to 1/10 FP and 1/20 FP than the other groups. Supplementation with βG reorganizes this toxic effect by mounting the AchE manners in the brain.

3.4. Effect of βG and / or FP on antioxidant parameters

Regarding the antioxidant status and oxidative stress, (Table 4) showed that, the βG supplementation had significantly highest level of GSH, with no significant divergences in MDA level in parallel to the CT. While the groups subjected to the both doses of FP were recorded a significant decrease in GSH with an expansions in MDA compared with the control group. The βG dietary supplementation improved the hazard effect of FP by increasing GSH and decreasing MDA level compared to groups that exposed to FP and fed on basal diet.

3.5. Effect of βG and / or FP on relative antioxidant genes expression

The supplementation with βG was resulted also in a significant increases in both GPx and GR gene expression compared with the control diet, without changes in CAT and SOD. Exposure to FP with the both doses resulted in significant decreases in the expression of all measured antioxidant genes. The βG co-treatment was soothed the FP risk impact by increasing the gene expression of CAT, SOD, GPx and GR enzymes in both FP concentration (Plate 1).

4. Discussion

Fipronil, a potent insecticide, produces very toxic effects in fish in a way similar to those produced in humans by damaging several enzymes and reducing fish immunity [52,53]. Thus, this study was directed toward finding a method to reduce these toxic effects using βG as immunostimulant to increase the immunity of *O. niloticus* to be able to face challenges of sub-lethal FP exposure.

The immunostimulant consequences of using βG in reducing the sub-lethal toxicity of FP. All growth parameters were observed to be reduced in FP-exposed groups when compared to the control group. The finding that supplementation with βG either alone or with FP successfully improved the growth parameters, especially at low concentrations of FP (1/20), confirms the highly beneficial effect of βG in prosperous the immune system of fish to enable them to better tolerate FP toxicity. The mechanisms of how FP induces its toxic effects and how βG improves the immune state were illuminated by considering the effects of these agents on immunity (IgM and lysozyme) and the antioxidant state. Both of IgM and lysozyme are good markers of immune status in fish; FP was found to decrease the serum levels of IgM and lysozyme, chiefly at high doses (1/10), which may explain the stunted growth of the tilapias. This finding also verifies the reports of Ref. [52] who implemented experiments on *Cyprinus carpio*. Similar results were also obtained by Ref. [53] who located that exposing *C. carpio* to 0.65 mg/l of FP for 7, 30 and 90 days had very toxic effects on the fish, as indicated by significant reductions in the levels of antioxidant enzymes and immunity. The improvements in growth parameters observed in the βG -supplemented groups can be attributed to the potentiating effects of βG on IgM and lysozyme, as observed in our results and as confirmed in previous similar studies by Refs. [54,55] on shrimp larvae; these researchers suggested that improvements in growth performance might be due to increases in the ability of shrimp larva to prevent infection and resist diseases. In addition, feeding low doses of βG to large yellow croaker expansions the level of lysozyme in fish serum, thereby increasing the ability of the fish to resist assorted diseases [16,56]. Other reports have certified the amendment in growth performance to other ways, such as the beneficial effects of βG in increasing the energy of fish [57]; improvements in nonspecific immunity through direct macrophage initiation [58]; recoveries in the production of both macrophages and neutrophils, as observed in channel catfish [59]; and advancements in the production of lysozyme, as perceived in Atlantic salmon, turbot and rainbow trout [60,61].

Regarding tilapias health status, FP was found to increase serum levels of ALT and cortisol, reflecting the deleterious effects of FP on the liver and on the general health state of the body. ALT is an indicator enzyme of liver status and is very sensitive to pollutant toxicity [62]. The increase in ALT level is an indicator of hepatotoxicity [63] caused by FP [64] conveyed that the levels of serum cortisol were increased in *C. carpio* exposed to 0.0428 mg/l FP for 45 days. Supplementation with βG reduces the toxic effect of FP, and this effect was observed through the improvements in serum ALT and cortisol levels in the groups treated with βG. This effect may also be attributed to the positive effect of βG in improving the immunity of fish to an extent that they could resist the effects of FP. Several studies have reported that administration of immunostimulants such as βG causes positive deviations in nonspecific immune responses of fish against infection, stressors, and toxicity [65].

LDH level are also generally increased when fish are exposed to stress or pollution [66]. In this study, the groups that were exposed only to sub-lethal doses of FP showed significantly higher level of LDH than the other groups, while feed supplementation with βG caused

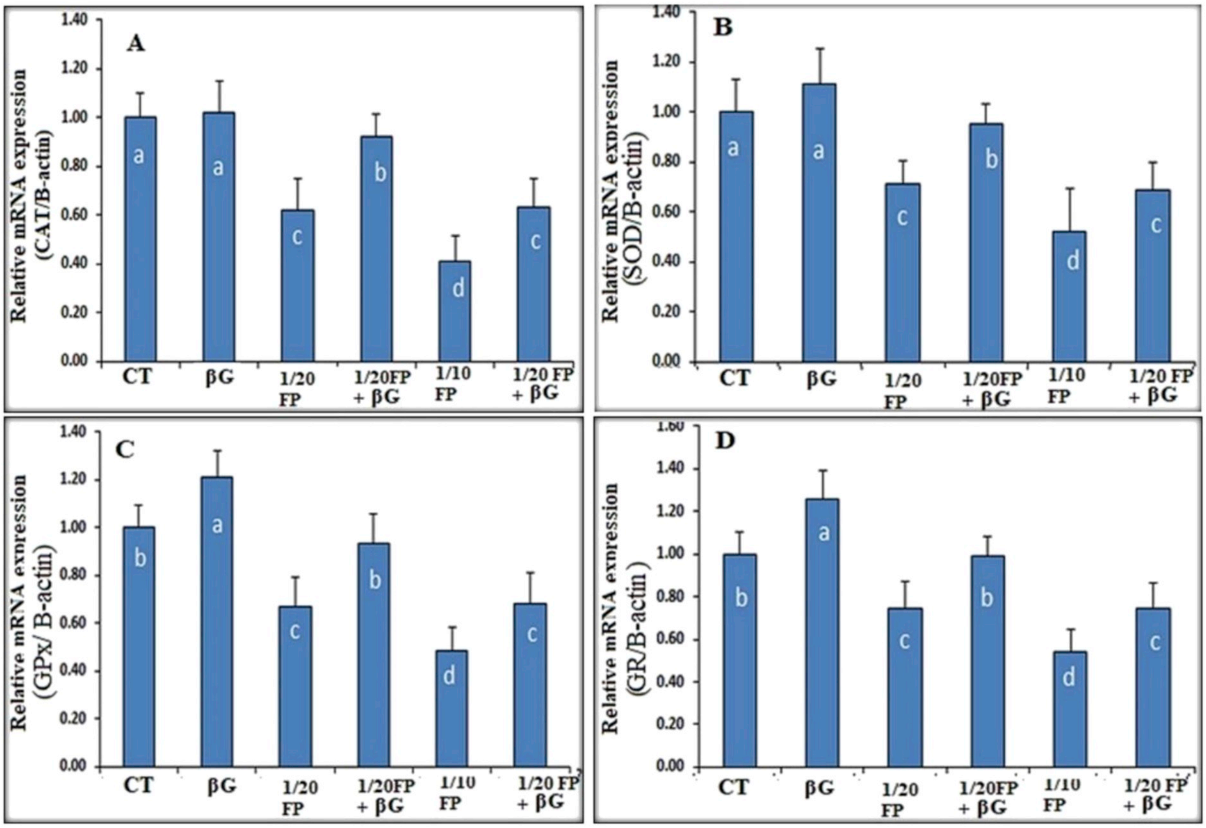

Plate 1. Effect of dietary supplementation with 0.4% βG on mRNA expression of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) of *O. niloticus* exposed to FP (1/20 and 1/10 96-h LC_{50}) (Figs. A–D).

significant decreases in the levels of LDH. Groups exposed to sub-lethal doses of FP also showed significant increases in the levels of MDH, which reflected to a tricarboxylic acid (TCA) cycle intermediary. The increased levels of MDH were due to the increased energy requirements of the fish under conditions of stress. On the other hand, the tilapias supplemented with βG showed significant decreases in the levels of MDH. The same results were observed regarding the levels of FDPase. Feeding fish diets supplemented with βG enhances liver function and increases transcription of pyruvate kinase while reducing F-ATPase, succinate dehydrogenase (SDH) and MDH expression [67].

Upon studying the effects of FP on fish brains, it was observed that AchE level was significantly decreased in the groups exposed to sub-lethal dosages of FP. AchE is reflected one of the greatest important pollution marker enzymes. Acetylcholine is formed in nervous tissue and is rapidly destroyed by AchE, a member of the cholinesterase family of hydrolytic enzymes [67]. Hydrolysis of acetylcholine by cholinesterase then drop the activity of cholinesterase. Reduced activity of AchE is an indicator of stress exposure in fish and results in the accumulation of acetylcholine [68] perceived that the existence of acids hinders AchE in tilapia. Additionally, sustained that inhibition of the secretion of AchE in *Cirrhinus mrigala* under stress conditions, such as overpopulation, caused the accretion of acetylcholine. In this study, the groups fed diets supplemented with βG showed significant improvements in the levels of AchE [69].

At the cellular level, antioxidants are accountable for shielding cells from damage by scavenging free radicals [70]. FP treatment was found to cause hepatic cell damage, as perceived by increases in serum ALT. This may be due to FP-induced increases in lipid peroxidation (as implied by MDA level) and reductions in GSH level along with an inhibitory effect on the expression of antioxidant hepatic genes enzyme CAT, SOD, GPx and GR. According to Ref. [24], MDA is inversely correlated with GSH, and FP additionally condenses GSH levels, initiating more oxidative stress in fish. Supplementation with βG improves the antioxidant state in cells by significantly diminishing MDA level, increasing GSH level, and increasing the expression levels of antioxidant enzymes, which aids in free radical scavenging and thus rallies the total health state. The upsurge in GSH level in this study might have been owing to the shielding effects of βG on the sulfhydryl unit of GSH [71].The dominant antioxidant force of βG may be due to its capacity to scavenge free radicals and hinder lipid peroxidation, thereby enhancing endogenous antioxidant capabilities and blocking free radical generation [72,73].

5. Conclusions

With the expressed findings, it is fulfilled that FP, a forceful insecticide, instigates critical health problems in *O. niloticus,* via negative impacts on immune status and reductions in the antioxidant state, emerging oxidative stress. The diminutions in immune status and the emerged oxidative stress cause reductions in growth parameters and general health problems. Supplementation with βG reform the immune state and raises antioxidant levels to profitably recover general health and growth performance; therefore, βG supplementation may have an encouraging effect in the tilapias industry.

Conflicts of interest

None.

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