β-1,3-glucan Improved Health Status, Stimulated Immunity, and Neutralized Lead and Fipronil Pollutants Toxicity of Nile Catfish (Clarias gariepinus)

Gamal A. Elmowalid (gaabedallah@vet.zu.edu.eg)
Zagazig University
Wael A. M. Ghonimi
Zagazig University
Hossam M Abd Allah M Abd Allah
Zagazig University
Abdelhakeem El-Murr
Zagazig University
Haytham Abdallah
Zagazig University
Ashraf M. Abdelwahab
Zagazig University

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Abstract

This study was designed to examine the impact of dietary β-1,3-glucan immunomodulatory and mitigating activities against fipronil and lead-induced pollution in African catfish (*Clarias gariepinus*) culture. Two hundred forty catfish were randomly divided into four equal groups: the first served as a control; the second was supplemented with β-1,3-glucan; the third was exposed to fipronil and lead; and the fourth was exposed to a combination of fipronil, lead, and β-1,3-glucan. The health status, haematological, immunological, and histopathological changes were all evaluated. Swelling on the dorsolateral side, spinal column deviation (scoliosis), sluggish movement, skin bleaching, excessive mucus secretion, significant variations in blood indices-related measures, and a 45% death rate were all observed in the third group. There was also a significant decrease in IL-1, IL-2, IL-6, and IgM concentrations, as well as a down-regulation of their corresponding gene expression. In the spleen, there were notable necrotic foci and hemosiderosis; in the intestine, severe enteritis and mucinous degeneration of the lining epithelium were common pathological findings. Fish treated with β-1,3-glucan alone or in combination with fipronil and lead demonstrated improved physiological activity, blood indices, and elevations in IL-1, IL-2, IL-6, and IgM concentrations, as well as up-regulation in their gene expression in splenic tissues, when compared to the third group. The spleen and intestine had normal histological architecture with 5% mortalities. No fish mortalities were observed in the control and β-1,3-glucan alone treated groups. These findings suggest that β-1,3-glucan could be a promising dietary supplement for catfish health, immunity, and as a fipronil and lead pollutant neutralizer in catfish culture.

1. Introduction

In developing countries such as Egypt, fish meat is a good source of protein from both a practical and economic standpoint [1]. The ecosystem and food additives used in aquaculture systems influence the quality and safety of fish and fish meat. Water pollutants in fish farming systems are regarded as major impediments to the development and sustainability of ecosystems and the fish industry. Climate change, nutrient shifts, acidification, habitat loss, exploitation, biological invasions, and chemical contamination are among the various threats to freshwater ecosystems that act as stressors and cause biodiversity losses [2]. Insecticide use in urban and agricultural areas is a significant source of chemical pollutants and stressors that can be harmful to other organisms in aquatic ecosystems [3] [4] [5] [6]. Insecticides can be transported into urban streams and waterways via irrigation runoff and storm water, even if they are not applied near surface water bodies, posing a significant threat to the fish industry [7]. Fipronil is a broad-spectrum insecticide that is widely used in agricultural and veterinary fields because of its efficacy at low doses [8], despite its adverse health and environmental effects. Its mechanism of action is to inhibit excitable membranes by targeting the γ-aminobutyric acid (GABA) receptor chloride complex. Fipronil's blockage of GABA-gated chloride channels reduces neuronal inhibition, resulting in hyper-excitation of the target host's central nervous system, convulsions, and, eventually, death [9] [10]. Several reports of Adverse Drug Experiences (ADEs) associated with the use of fipronil-containing products have been reported in target and non-target animal species (honeybees, fish, aquatic invertebrates, and upland

game birds), as well as in humans either applying the product or handling the target animal after application. According to the United States Environmental Protection Agency (US EPA), fipronil is highly toxic to fish, such as bluegill sunfish (LC 50 = 0.083 ppm) and rainbow trout (LC 50 = 0.246 ppm). Fipronil was found to be toxic to Nile tilapia (*Oreochromis niloticus*) at a dose of 42 g/L [11]. Fipronil caused severe toxicity in zebrafish embryos by disrupting higher-level genomic DNA methylation, which is involved in cell signalling and development [12]. Furthermore, fipronil metabolites, such as sulfone and sulphide, are more toxic than fipronil itself [13]. Also, it was reported that fipronil is highly toxic to both *Aristichthys nobilis* fish and *Musca domestica* insects [14].

Because of its low rate of elimination, lead (Pb) is one of the most dangerous pollutants in the environment that can accumulate in the body [15]. It enters aquatic systems through urban, mining, and agricultural runoff, atmospheric precipitation, lead-containing fertilizers and gasoline, and a variety of other routes [16] [17]. Lead has been shown in studies to cause neurological, haematological, gastrointestinal, reproductive, circulatory, immunological, histopathological, and histochemical changes, as well as a variety of other undesirable effects in a dose and time-dependent manner [18] [19] [20]

Prebiotics have been shown to be effective in both human and animal feeding practices. They have recently gained attention in aquaculture for disease control and competition with various environmental stressors, as well as to promote the growth of cultured fish. β-Glucans are the most commonly used prebiotic in aquaculture, and they have been widely used to reduce the negative effects of stress and improve various physiological parameters such as growth and feed conversion rates in a variety of fish [21]. β-glucan increased the food conversion rates of *Labeorohita* fingerlings' growth [22] *Pagrus auratus* snapper growth [23] and improved rainbow trout survival and resistance [21]. β-glucan was discovered to increase the production of crap antibodies [24] [25] post *Aeromonas hydrophila* challenge. Rainbow trout's complement fraction 3 (C3) gene expression was altered, and inflammatory cytokines gene expression were up-regulated after β-glucan bath immunostimulation [14].

Even trace amounts of fipronil insecticide or lead heavy metal entering waterways can act as stressors, disrupting the ecosystem and resulting in growth retardation, immunosuppression, and increased mortalities in catfish. In this scenario, and to overcome pollutants' toxicity, we hypothesize that adding immunomodulatory agents such as β-1,3-glucan to the diet could neutralize lead and fipronil stressors' activities and strengthen catfish defence mechanisms. This would have been a cost-effective way to improve health and prevent microbial infection outbreaks.

2. Materials And Methods

2.1. Chemicals:

2.1.1. Fipronil: Fipronil 20% was obtained from Yongnong Biosciences Co., Ltd. China**.** Technical grade Fipronil (C12H4Cl2F6N4OS) (99.1% pure) was manufactured by Bio Quest International Private Limited, Mumbai, India, and it consists of two isomers at a ratio of 50:50. Fipronil’ doses were estimated [26].

2.1.2. Lead: The solid white crystal form of lead nitrate Pb(NO3)2 was obtained from Fisher Scientific Company in Canada.

2.1.3. Beta glucan: β-1, 3-glucan (Sigma, U.S.A.) was dissolved in phosphate buffered saline (PBS), thoroughly mixed, and subsequently added to basic diet constituents at a rate of 0.1% before palletization. The feeding period and β-1,3-glucan dose used in this study were based on the earlier report [27].

2.1.4. Cytokines and antibodies: Fish Interleukin-1β (IL-1β), ELISA Kit, Cat. No. MBS700023 and Fish Interleukin 6, (IL-6) ELISA Kit, Cat. No. MBS015740 were used in this study for the detection of IL-1β and IL-6 levels. The kits were obtained from MyBiosource Co. (San Diego, California, USA). The Immunoglobulin M (IgM) ELISA Kit. Catalog No. CSB-E12045Fh, was purchased from CUSABIO BIOTECH CO., Ltd. and it was used to quantitatively determine the IgM level.

2.2. Fish management and maintenance

Two hundred forty Nile catfish with an initial 40 ± 3.0 g body weight (mean ± SD) and 14.71 ± 1.23 cm of fork length were used in this study, and they were obtained from a local hatchery (Abbasa, Ash Sharqiah, Egypt). The fish were transferred to the Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Zagazig University, Egypt, where the experiments were performed. Initially, fish were immersed in a salt bath of NaCl at a concentration of 2.5% for five minutes to get rid of external parasites and fungal infections. The fish were acclimatized for two weeks before starting the actual experiments, which lasted a total of two months. During the acclimatization period, fish were fed a commercial pelleted feed containing 32% protein and were placed in glass aquaria (80 x 40 x 30 cm capacity). Each aquarium had 60 litres of chlorine-free tap water. An aerator was used to provide air supply; a thermostatically controlled heater kept the water temperature at 26°C; the pH of the water was kept at 6.5-7.0; dissolved oxygen averaged 60.5 mg L-1; ammonia averaged 0.01 mg L-1; and nitrite was 0.20 mg L-1. These parameters were measured routinely with a freshwater kit (La Motte®, Chertestwon, MD, USA). Approximately 30% of the water was changed daily, and a constant water flow was maintained.

2.3. Experimental design

After the acclimatization period, the 240 catfish were divided into four experimental groups (60 fish per group, each group in triplicate) after acclimation and then placed into 16 aquaria at random (4 aquaria per group with 15 fish per aquarium). The first group served as a control, and the fish were fed a basic diet without any additions or treatments, while the fish in the second group were fed a basic diet supplemented with 0.1% of β-1, 3-glucan, and the fish in the third were fed a basic diet and exposed to a combination of lead nitrate at 0.041 mg/L (1/10 96 h LC50) according to the New Pesticide Fact Sheet (1996) and fipronil at 2.8 mg/l (1/10 96 h LC50) according to Mahmoud (2013). The fourth group of fish received a basic diet supplemented with 0.1% of β-1, 3-glucan before being exposed to lead nitrate and fipronil at the same mentioned concentrations. All of the fish in the various experimental groups were fed their individual diets ad libitum four times daily at a rate of 3% of their body weight. Every day, the fish

were checked for any changes in their general health, clinical signs, odd behavior or coloration, or respiratory distress. The affected fish's post-mortem lesions and mortality rate were documented.

2.4. Molecular determination of IL-1β, IL-2, or IL-6

Spleens were obtained from dead or euthanized fish, quickly maintained in liquid nitrogen and stored at -80 C to be used in a semi-quantitative RT-PCR [28] using the appropriate specific set of primers to measure IL-1, IL-2, or IL-6 cytokine gene expression (Table 1). RNA was extracted from various fish groups using the Gene JET RNA purification kit (Fermentas, UK) according to the manufacturer's instructions. A Nano-Drop ®ND-1000 Spectrophotometer (Wilmington, Delaware, USA) was used to verify the quantity and purity of the RNAs collected. The reverse transcriptase enzyme Superscript II RNase H (Invitrogen, Carlsbad, CA, USA) was used to make cDNA. To express arbitrary units of relative abundance, the values of the specific targets were normalized to those of β-actin. After amplification, PCR products were run on a 2% agarose gel in 90 mM Trisborate, 2 mM EDTA buffer (TBE), pH 8, and stained with ethidium bromide and observed by UV transillumination. To check the size of amplification products, a DNA ladder molecular weight marker (Gel Pilot 100 bp ladder (Cat. No. 239035) supplied by (QIAGEN, USA) was employed. Consequently, the product densities were analyzed by the gel documentation system (Bio Doc Analyze, Biometra, Germany) and photographs were taken using a Sony XC-75 CE camera (VilberLourant Inc. Cedex, France), with the density of the bands assessed using Photo-Capt v.99 Image software (VilberLourant Inc. Cedex, France).

2.5. Immunological and biochemical parameter evaluation

At the end of the feeding experiment, ten randomly selected fish from each group were collected and sedated with tricaine methane sulfonate MS-222 (100 ppm) for caudal vein blood sampling. Each blood sample was divided into two aliquots. One aliquot was placed in EDTA-containing tubes and was utilized for haematological analysis according to Feldman et al., (2000), and the second aliquot was allowed to clot at 4 C and centrifuged at 1500 x g to separate the sera, which were then aliquoted and stored at -20 C for ELISA assay. IL-1 and IL-6 cytokines, as well as immunoglobulin (IgM) levels in the various test groups, were measured, as well as some biochemical parameters. Both ELISA and biochemical assays were carried out as directed by the kits' manufacturer.

2.6. Tissue sampling

Intestinal and splenic tissues were obtained before and after the catfish treatments with the tested compounds in the four groups. Splenic tissues were employed for both histopathological and molecular detection of the target inflammatory cytokines' gene expression. Intestinal tissues were used for histopathological investigations.

2.7. Histopathological investigations

Specimens from the intestine and spleen were collected and immediately fixed in a 10% buffered neutral formalin solution for 48 hrs, then processed histologically, where the specimens were dehydrated in

ascending grades of ethanol (70-100%), cleared in xylene, and finally embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with Hematoxylin and Eosin (H&E) dyes for any histological changes according to [28]. The microphotographs were taken using a digital Dsc-W 130 super steady cyper shot camera (Sony, Japan) connected to an Olympus BX 21 light microscope.

2.8. Statistical analysis

SPSS version 20 was used to perform statistical analysis on the data. Statistical packages (IBM 1, New Orchard Road, Armonk, New York 10504-1722, United States) presented as a mean ±SD, n = 10 were used. Statistical differences among groups were performed using a one-way analysis of variance (ANOVA). Duncan's test was used to test inter-group homogeneity. The level of statistical significance was set at $p \leq 0.05$.

3. Results

The mortality rate in the different catfish (*Clarias gariepinus*) groups

The third group of catfish (*Clarias gariepinus*) subjected to lead nitrate and fipronil had the highest mortality rate of 45%, with erection in both dorsal and pectoral fins (Figure 1). Meanwhile, the mortality rate in the fourth group, which received β1,3-glucan plus a mixture of fipronil and lead nitrate, was 5% without any abnormalities in the fins. In the meantime, neither the fish in the untreated control group nor the fish in the -1,3-glucan supplemented group died.

Clarias gariepinus's health status

The third group of catfish subjected to fipronil and lead nitrate experienced a slow escape reaction and respiratory discomfort, as well as a loss of body weight, after being exposed to fipronil and lead nitrate. Furthermore, abnormal semi-circular swimming behavior and slow movement, as well as expansion of the dorso-lateral side and lateral deviation in the vertebral column, were seen. Skin abnormalities such as bleaching, pale coloration (Figure 1), and fish weakness predominated in the same catfish group, and fish death was linked to air bubbles and excessive mucus secretion. In the fourth group of catfish that were treated with β-1,3-glucan and subjected to fipronil and lead nitrate, improved physiological activities such as active escape response to capture, no respiratory distress, no clinical signs, and normal body weight were observed. The fish in the second group that received β-1,3-glucan, on the other hand, showed the highest physiological health activities as well as a substantial increase in fish weight among the four investigated groups. The fish in the untreated control group, on the other hand, showed no abnormal behavioral or clinical abnormalities and normal body weight.

Changes in haematological indices, IL-1 and IL-6 levels, and immunoglobulin M levels.

At the completion of this study, various blood indices were evaluated to determine the impact of lead and fipronil pollutants on Nile catfish. Table 2 shows that a combination of both pollutants (third group) significantly reduced all of the haematological parameters studied. When compared to the β-1,3-glucan

non-supplemented control group, fish supplementation with β-1,3-glucan, either alone (second group) or in combination with fipronil and lead nitrate (fourth group), resulted in a significant increase in the target blood parameters.

The second group of β-1,3-glucan supplemented catfish exhibited significantly greater plasma levels of IL-1 and IL-6 cytokines, as well as IgM, compared to the control catfish. In comparison to the first and second groups, the catfish in the third group that were exposed to lead and fipronil had much lower levels of IgM, and IL-1 and IL-6 cytokines. In catfish of the fourth group supplemented with β-1,3-glucan and subjected to a combination of fipronil and lead nitrate, however, both IL-1 and IL-6 cytokines, as well as IgM levels, were slightly reduced, and their concentrations were close to the control catfish concentration levels (Table 2).

IL-1β, IL-2 and IL-6 cytokine gene expression in the tested groups.

Semi-quantitative PCR was used to evaluate the expression of pro-inflammatory and inflammatory cytokine genes in splenic tissues. Figure 2 shows that the catfish supplied with β-1,3-glucan had the highest expression levels of IL-1β, IL-2, and IL-6 expression, followed by the control non supplied group. The third group (Lane 3), which was exposed to fipronil and lead nitrate induced pollution had significantly showed lower levels of IL-1β, IL-2, and IL-6 gene expression. After β-1,3-glucan supplementation, the fourth group that received fipronil and lead nitrate had IL-1β, IL-2, and IL-6 gene expression levels (lane 4) that were comparable to the control and β-1,3-glucan supplemented groups (lane 1 and 2, respectively) as shown in Fig. 3.

Histopathological findings

The intestine and spleen of the four groups were examined to see if there were any histopathological alterations. In both the control and β-1,3-glucan supplemented groups, the intestine showed normal intact mucosa, submucosa, muscular coat, and serosa (Fig. 4.1), and the spleen showed normal white and red pulps (Fig. 5.1). Meanwhile, the Fipronil and lead-treated group showed severe enteritis in the form of edema, severe blood vessel dilatation and congestion with numerous lymphocyte infiltrations in the submucosa (Fig. 4.2). Also, mucinous degeneration in the lining epithelium was observed (Fig. 4.3). The splenic tissues showed severe depletion and necrosis in the lymphocytes of white pulp and hemorrhagic red pulp (Fig. 5.2), severe congestion in the splenic blood vessels (Fig. 5.3), and some examined sections showed severe necrosis of white pulp with hemosiderosis of brown pigments; excessive accumulation of iron deposits (Fig. 5.4). While, fish intestine and spleen of the fourth group that received fipronil and lead and supplemented with β-glucan showed mild histopathological alterations in their architecture. The intestine showed intact mucosa and a slight increase in the goblet cells without any inflammatory cells in the submucosa (Fig. 4.4). The spleen revealed normal white and red pulps with activated melano macrophage centres (Fig. 5.5).

4. Discussion

Exposure to heavy metal pollutants could impair fish's ability to smell, affect swimming performance, reduce metabolism, damage vital organs, and could induce immunosuppression that may result in increased susceptibility to disease and mortality. It is, therefore, important to enhance tolerance against water pollutants such as lead and fipronil that could target fish culture through waterways or the surrounding environment. Functional feeding is an emerging paradigm in the aquaculture industry that aims to develop diets of balanced nutrition supplemented with feed additives to improve both the health and disease resistance of cultured fish [30]. In this paper, we looked at the effects of stress on Nile catfish (*Clarias gariepinus*) exposed to fipronil and lead nitrate, as well as the role of dietary β-glucan feed additives in mitigating their negative effects. The choice of Nile catfish was based on its tremendous ability to withstand environmental and aquatic stresses and its high commercial value in Egypt as one of the major economic protein sources. β-1,3-glucan was used in this study by a dose of 0.1% [27] to avoid the consequences of higher dosage side effects. A higher dosage (2%) did not reduce the induced cold-stress fish mortality, whereas a dosage of 0.5% resulted in significantly lower mortality [31]. Other studies suggest that the effect of β-1,3-glucan on stress relief is affected by dose and duration of the experiment. B-1,3-glucan overdoses could even induce immunosuppression [32] [33] [23]

Catfish that received fipronil and lead simultaneously showed clinical signs of semi-circular swimming behaviour with some dorsal swellings and vertebral column deviation, indicating poor physiological conditions and explaining the lack of escape catch response. Fipronil has been proven to disrupt the central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels, components of the central nervous system that result in muscle exhaustion, nervous manifestation, and respiratory distress [34] [35], which could explain the clinical signs and the high mortality rates observed in fish. A study showed that lead at a concentration of 3.2 mg/L was associated with loss of balance and reduced activity, which could be another explanation for the semi-circular sluggish movement and the slow escape reflex seen in our study [36]. They also reported that lead induced skin bleaching and a thick layer of mucus covered the fish skin, which supports the results obtained in the current study. The overall health and physiological status data were in agreement with the earlier results [37] and with the recent results obtained by [26], who mentioned that fipronil caused mortality in different fish species like rainbow trout, bluegill sunfish, and Nile tilapia, with 96 hr LC50 of 0.246 mg L/1 and 0.083 mg L/1, respectively. It was suggested that administration of β-1,3-glucan as a dietary supplement at a concentration of 0.1% [27] would ameliorate the undesirable toxic effects of fipronil and lead in catfish. Supplementation of β-1,3-glucan resulted in significant improvement of the overall fish health parameters and a lowering of mortality rates to 7% compared to 45% over the six-week study period. No weight loss (data not shown), abnormal movement, skin discolouration, or anatomical deviation in the vertebral column were observed in the groups that received β-1,3-glucan in the diet, confirming that it can ameliorate the detrimental effects of pollutant stress.

Chemicals and insecticides used in aquaculture systems act as stressors, increasing susceptibility to infections and disease outbreaks while limiting aquaculture production and trade. We believe that activation of fish immune system functions would be associated with increased protection against infectious diseases. Therefore, a major objective of this study was to strengthen catfish immunity in order

to prevent secondary microbial infection. Herein, the stress of fipronil and lead nitrate administration in catfish remarkably lowered IgM and IL-1β and IL-6 levels. Supplementation of β-1,3-glucan to the diet significantly increased fish IL-1β and IL-6 cytokines and IgM to higher levels compared to those observed in the control, and this supports the earlier findings [14] [24] [38] [39] in other fish species, confirming the immunomodulatory activity of β-glucans. The exact mechanism of triggering cytokines and IgM secretion is not fully established. *In vitro* studies indicated that β-glucan elicited pro-inflammatory cytokines in activated macrophages [40]. However, feeding β-glucan to fish hardly improves the production of total or specific immunoglobulin, as reported in Nile tilapia (*Oreochromis niloticus*) [41], common carp [42], rainbow trout [43] and gilthead sea bream [44], which is contradictory to our results. This contradiction may be attributed to fish species, feeding regimen, or β-glucan dosage used by nature. The elevated levels of the catfish major cytokines and the main protective and neutralizing antibody, IgM, reported in this study could explain the improved resistance against different aquatic pathogens in the different fish species after supplementation with β-glucan as reported in various studies [29] [45] [46] [46]. Our results also showed that β-glucan feeding enhanced the up-regulation of immune-related cytokine gene expression in catfish, confirming their serum higher levels.

Conclusion

The findings highlight the tremendous potential of β-1,3-glucan and open up the possibility of using it as a fipronil and lead pollution neutralizing agent as well as an immunomodulator in fish culture. Research reports have shown some contradiction in β-1,3-glucan dosages, route, and time of administration and duration of supplementation in enhancing various parameters related to physiological status, survival, and immunity. More research on the unexplored roles of β-1,3-glucan should be conducted in order to determine the precise mechanism of action and to use β-1,3-glucan as a potent prebiotic in aquaculture.

Declarations

1. Ethical Approval

All methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments. The study was carried out in accordance with the guidelines and regulations approved by the Institutional Animal Care and Use Committee of Zagazig University, Ash Sharqiah, Egypt.

2. Consent of publication

At the time of submission, the authors declare that the manuscript had not been submitted to any other journal.

3. Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

4. Declarations Competing interests

The authors declare that they have no competing interests.

5. Funding

The authors confirm that this study has not been funded by any public or private agency.

6. Author contributions

All of the authors meet the authorship criteria with the following contributions specified: GE conducted most of the literature search, designed the study, and contributed a major portion to the writing and revising of the manuscript. WG Conducted the histopathological studies and contributed in writing the manuscript and supervised the paper. HMA, HA, AE, and AA carried the molecular, biochemical and immunological studies, reviewed and supervised the paper. All authors read and approved the final manuscript.

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Tables

Tables 1 and 2 are available in the Supplementary Files section

Figures

Figure 1

Gross examination of catfish treated with lead and fipronil and supplemented with β-1,3-glucan (A) versus catfish treated with lead and fipronil (B). Lateral deviation of the vertebral column (blue arrows); skin bleaching; with haemorrhage in the lateral fins (black arrows) in catfish in the group exposed to fipronil and lead nitrate, whereas those treated with lead and fipronil and supplemented with β-1,3-glucan showed no gross abnormalities.

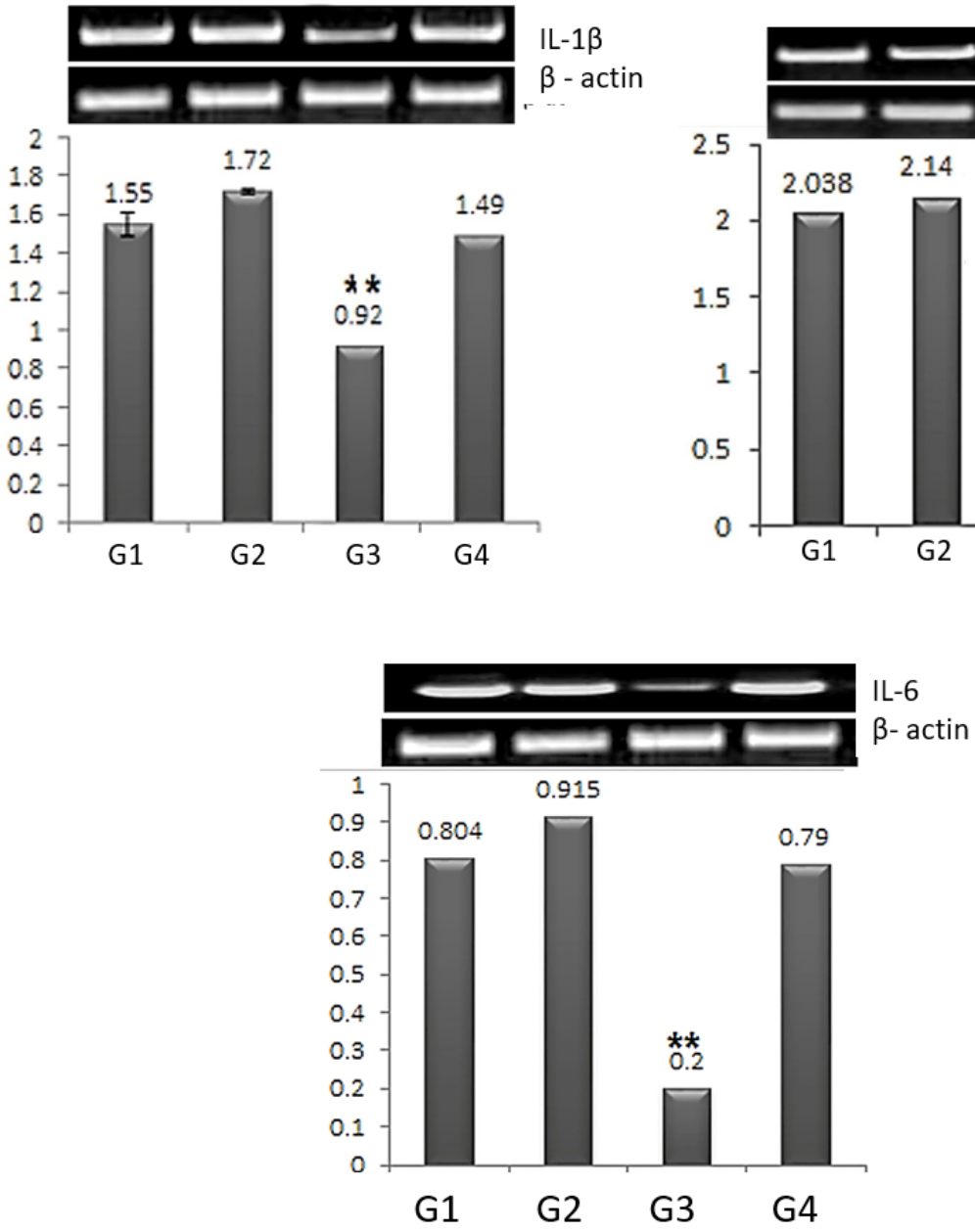

Figure 2

Analysis of relative gene expression of IL-1β, IL-2 and IL-6 in relation to β-actin after lead, fibronil and β-1,3-glucan treatment in

splenic tissues of *Clarias gariepinus*

** indicates high level of significance (p-value is less than 0.01)

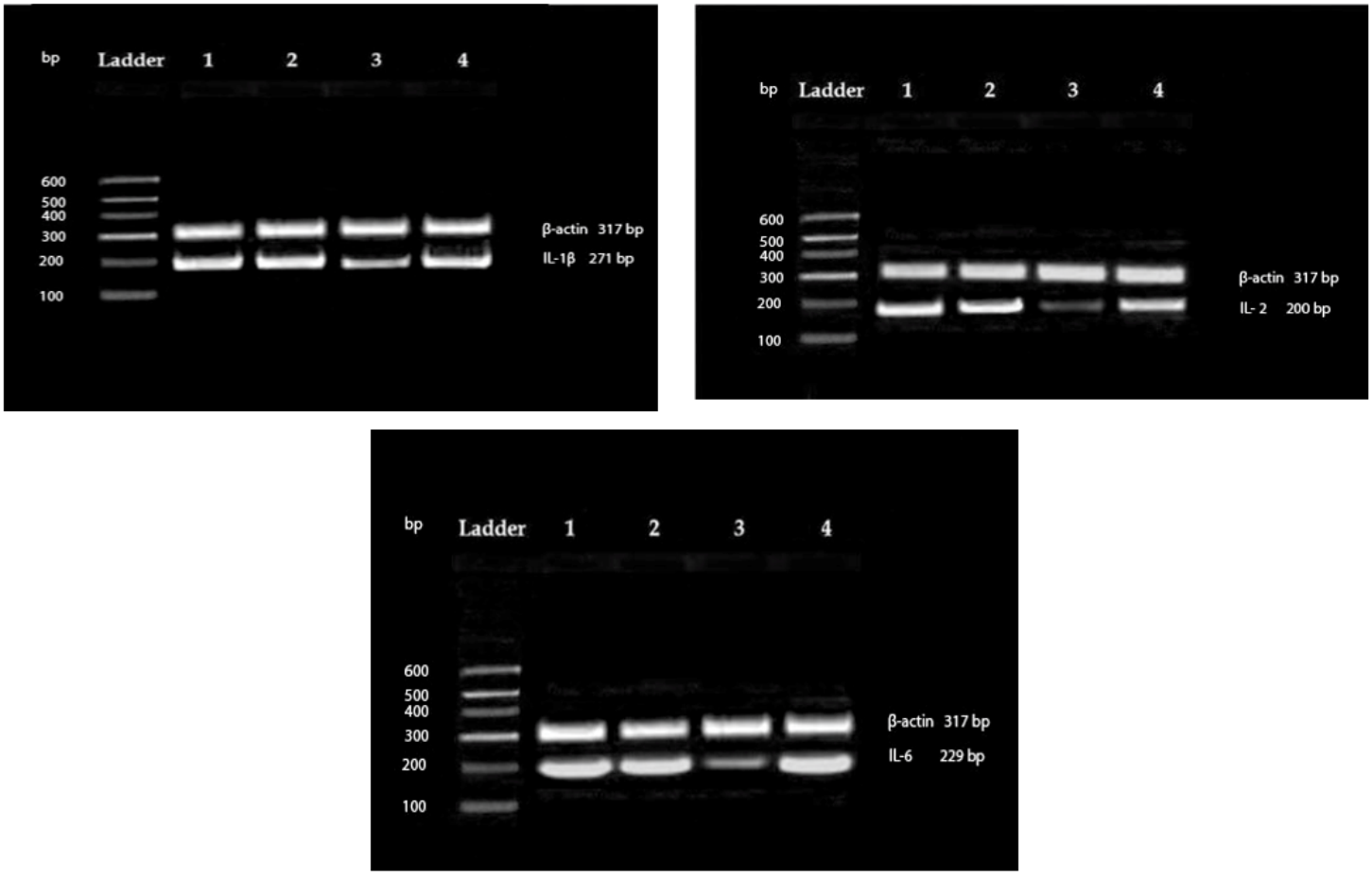

Figure 3

Semi-quantitative PCR of IL-1β, IL-2 and IL-6 cytokines in the experimental catfish groups. The third group (Lane 3), which was exposed to fipronil and lead nitrate induced pollution had significantly showed lower levels of IL-1β, IL-2, and IL-6 gene expression. After β-1,3-glucan supplementation, the fourth group that received fipronil and lead nitrate had IL-1β, IL-2, and IL-6 gene expression levels (lane 4) that were comparable to the control and β-1,3-glucan supplemented groups (lane 1 and 2, respectively)

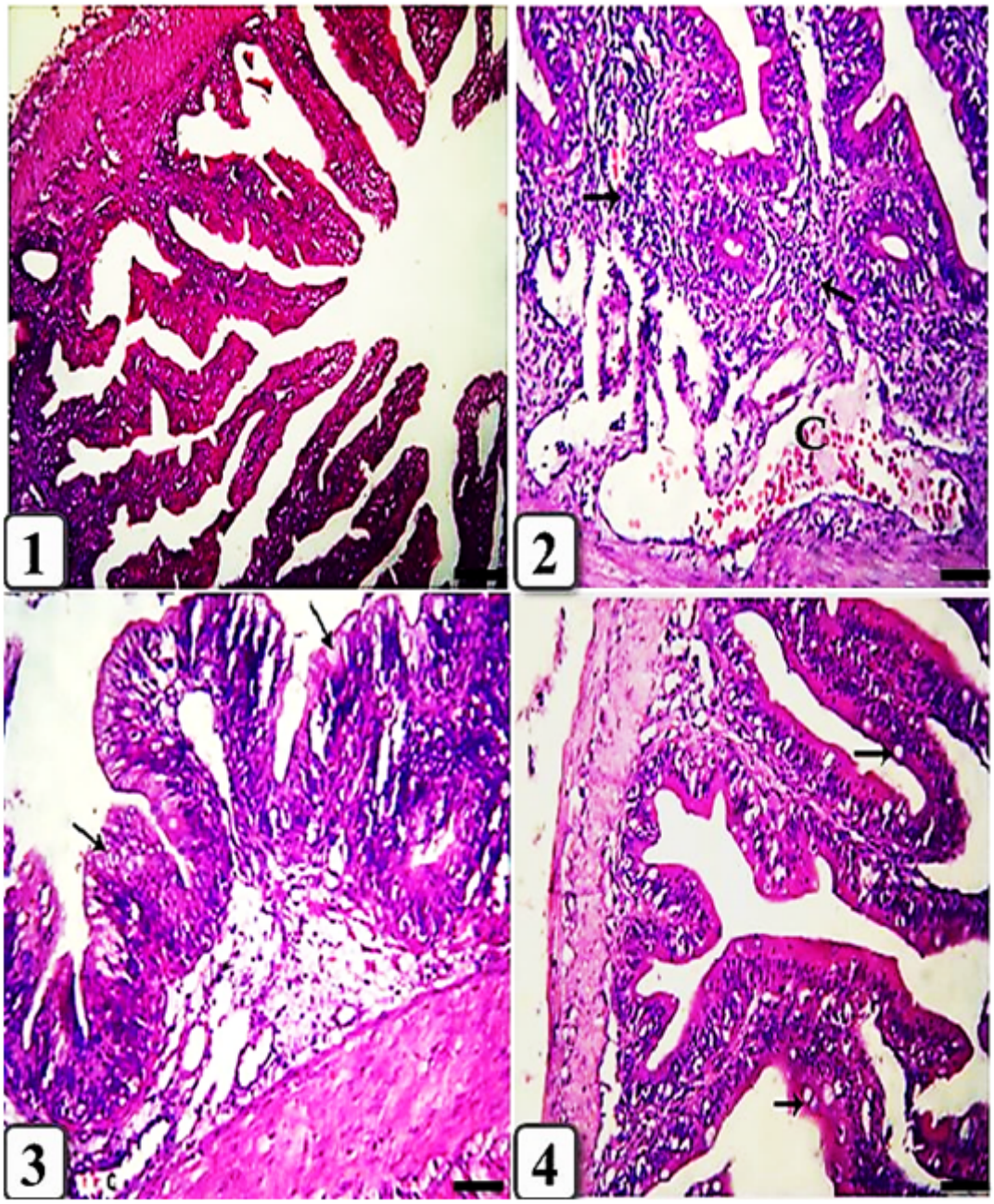

Figure 4

Histopathological effect of Lead, fipronil and β-glucan on the intestinal tissues of *Clarias gariepinus*

1)Control or β-glucan group showed normal, intact intestinal mucosa, submucosa, muscular coat and serosa.

2)Fipronil and lead treated group showed catarrhal enteritis with numerous lymphocytes infiltration (arrows) and sever blood vessels dilatation with congestion (C) in the submucosa

3)Fipronil and lead treated group showed mucinous degeneration of the lining epithelium (arrows) .

4)Fipronil and lead with β-glucan treated group showed intact mucosa and slight increase in the goblet cells (arrows) and without any inflammatory cells in the submucosa. **Stain** H&E x Scale bar = 50 µm.

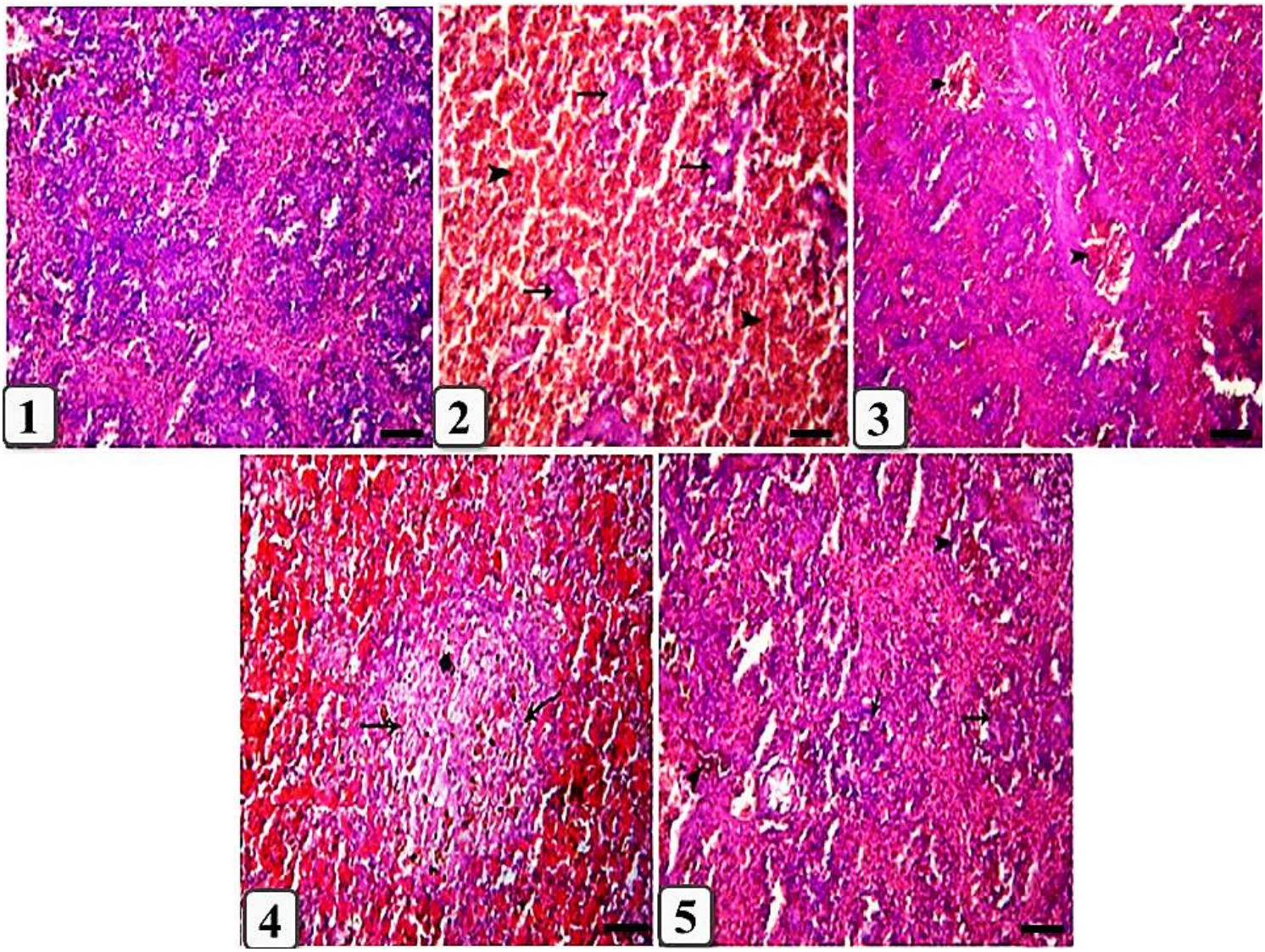

Figure 5

Histopathological effect of Lead, fipronil and beta glucan on the splenic tissues of *Clarias gariepinus*

1)Control or β-glucan group showed normal splenic tissues with normal white and red pulp.

2) Fipronil and Lead treated group showed severe depletion and necrosis in the lymphocytes of white pulp (arrows) and hemorrhagic red pulp (arrowheads).

3)Fipronil and Lead treated group showed sever congestion in the splenic blood vessels (arrowheads) .

4) Fipronil and Lead treated group of some examined sections showed severe necrosis in the lymphocytes of white pulp, hemosiderosis of brown pigments (arrows).

5)Fipronil and Lead with β-glucan treated group showed spleen with normal white (arrows) and red pulp and activated melanomacrophages centers (arrowheads) . Stain: H&E x Scale bar = 50 µm.

Supplementary Files

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