Chelonian Conservation And Biology

Vol. 19 No. 1 (2024) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2024.01(1).1983-1993

EFFECT OF *Nigella sativa* ON GROWTH, HEMATOLOGY AND LIVER ENZYMES (ALP, ALT AND AST) AGAINST LEAD INDUCED TOXICITY IN *Catla catla*

Saneela Kauser[1], Amara Akhtar[1], Muhammad Ayaz[1], Musfira Jabeen[1], Irsa Saleem[1], Irsa Shahab[1], Aqsa Matee[1], Muhammad Shoaib Khan[1], Muhammad Ahmad Saqib[1]

[1]Department of Zoology Wildlife and Fisheries, University of Agriculture Faisalabad, Pakistan.

Abstract

Aquaculture is the biggest fish production sector that meets the demands of fish globally. Lead (Pb) pollution from various human activities poses a significant threat to aquatic life. The present study explored the adverse effects of lead-based toxicity in *Catla catla* (*C. catla*) and the ameliorative role of *Nigella sativa* (*N. sativa*) supplementation. There were four treatments in which forty-eight fish were equally distributed. T_0 was the control group fed with a basal diet. T_1 was fed with *N. sativa (*10 g/kg*)*. T_2 was fed with 7 mg/L lead and T_3 was fed with lead (7 mg/L) and *N. Sativa* (10g/kg). The growth of *Catla catla* was assessed weekly to evaluate the impact of different treatments on weight gain and length. Lead and *N. sativa* Group (T3) showed improved growth (27.05±1.09) as compared to the lead group (20.90±1.18) which indicated that *N. sativa* supplementation mitigated some of the adverse effects of lead toxicity. Length gain was maximum in T1 (12.08±1.12) as compared to other groups. Hematology parameters like white blood cell (WBC), hematocrit, red blood cell (RBC), concentration of hemoglobin and platelets were measured through a hematology analyzer. RBC Count (1.33±0.09), hematocrit (23.81 ±2.62) and hemoglobin (5.12±0.05) decreased in the lead group. WBC count (47.83±0.86) increased in the exposed group as compared to other treatments. Biochemical tests for the liver which contain alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was performed through assay kits to assess liver toxicity. ALT (31.3 ±2.78), AST (57±2.70) and ALP Levels (27.2±2.48) increased in the lead group (T2) indicating significant liver damage due to lead toxicity. The T3 group showed lower enzyme levels ALP(23.6±1) ALT(28.5±2.06)and AST (53.3±1.74) compared to other treatments demonstrating the hepatoprotective effect of *N. sativa*. Statistical analysis showed that the results are significant ($p<0.05$).

Keywords: *Nigella sativa,* Hematology, Toxicity, *Catla catla,* Growth

Corresponding Author: Saneela Kauser (saneelakauser633@gmail.com), Amara Akhtar (amara.akhtar47@gmail.com), and Muhammad Ahmad Saqib (ahmadsaqib0913@gmail.com)

Introduction

Fish is a vital source of nutrition and food security in developing nations of the world (Cojocaru *et al.*, 2022). *C. catla* is one of the most cultivated freshwater fish as a result of its good taste, protein content, size and omega-3 fatty acids which are very important for the function of the brain. *C. catla* is the fastest-growing fish native to the riverine system in Pakistan, Nepal, Northern India, Myanmar and Bangladesh. It is a cost-effective choice for aquaculture and has the best market demand (Brraich and Kaur, 2022).

Aquaculture is the fastest-growing sector which plays a very important role in our economy. It is a source of animal protein so it is a need of time to increase the production of aquaculture to fulfill the demands of the overpopulation (Harikrishnan *et al.*, 2022). Aquaculture is recognized as a critical solution to global challenges including food security, malnutrition and poverty (Aloo *et al.*, 2017).

Heavy metal pollution is a severe threat to the aquatic environment and has devastating effects on aquatic life. Anthropogenic activities such as agriculture, landfills and wastewater are the primary sources of heavy metal accumulation in water bodies. Immediate actions should be taken to prevent further contamination and protect the aquatic ecosystems from irreversible damage (Shahjahan *et al.*, 2022). Contamination by heavy metals can cause damage to the liver, dysfunction of the kidney, metabolic disturbances and even death in extreme cases (Al-Busaidi *et al.*, 2011).

Lead is known as a toxic heavy metal which occurs naturally in the environment. It is discharged from various industries, lead dust, street runoff, agricultural fields and municipal wastewater which can directly enter the aquatic environment causing lead toxicity for aquatic organisms (Garai *et al.*, 2021). Lead poisoning in fish can cause an imbalance between antioxidants and pro-oxidants resulting in oxidative stress. Lead exposure can also harm the immune system causing various toxic effects. The research is crucial to determine the extent of lead exposure in fish (Ishaque *et al.*, 2020).

Lead poisoning can cause severe damage to white and red blood cells, leading to significant changes in blood parameters. This condition can also have harmful effects on the nervous system. Prolonged exposure to heavy metals like cadmium and lead has been categorized as toxic due to their ability to cause harm even at low levels. The physiological and biochemical characteristics of aquatic organisms living under pollution stress are essential in controlling the aquatic ecosystem and these characteristics serve as vital biomarkers (Abdel-Warith *et al*., 2020).

Medicinal plants in animal feed have been used to increase the growth of animals significantly (Chen *et al.*, 2016). They are a valuable source of natural and synthetic drugs, containing unique active compounds that can affect biological processes (Schuhladen *et al.*, 2019). Aquaculture has shifted towards plant-based supplements because of low cost and minimal negative effects on fish health and the promotion of sustainable practices. Plant-based diets with bioactive components increase growth, improve immunity and increase resistance to diseases (Reverter *et al.*, 2014). Medicinal plants can provide an alternative to synthetic drugs in aquaculture (Gabriel *et al.*, 2015).

Nigella sativa is a member of the Ranunculaceae family which contains several bioactive molecules including p-cymene, thymoquinone and carvacrol (Shahid *et al.*, 2017). Ancient herbalists referred to black cumin as the ‘herb from heaven’ acknowledging it as a miracle herb (Ahmad *et al.*, 2013). Thymoquinone is a phytochemical found in *N.sativa* with various medicinal properties contributing to the plant's overall health benefits (Woo *et al.*, 2012).

Hematological parameters like hematocrit value, RBC count and hemoglobin concentration are often used to evaluate the toxicity of lead in aquatic environments. Exposure to lead in fish can cause changes in these parameters by inhibiting the enzyme activities involved in heme biosynthesis. The exposure to high lead concentration is directly linked to a decrease in hematological parameters. This effect is observed among different species of cells (Kim and Kang, 2015).

The liver is the main organ to performs important functions such as biotransformation reactions, digestion, storage and energy metabolism. It is also the primary organ for removing toxic substances from the body making it a useful biomarker for evaluating toxicity. Because of its role in eliminating contaminants, the liver is often a target for the accumulation of toxins (Sharma and Jindal, 2020). *Nigella sativa* promotes growth, antioxidants and hepatoprotective and hematoprotective effects. Medicinal plants possess pharmacological properties and may be used in fish feed. Bioactive components promote health benefits, natural growth and immunostimulation. The study shows that few plants were tested in fish models against xenobiotic-induced toxicity (Latif *et al*., 2021).

Objectives

The aims of this study were as followed:

- Investigation of the efficacy of *N. sativa* seed extract on the growth of *C. catla*.
- Analysis of the effect of lead on the liver enzymes of *C. catla*.
- Examination of the hematological variations of *C. catla* exposed to lead.
- Studying the impact of *Nigella sativa* as a hepatoprotective and hematoprotective agent.

Review of Literature

Khalesi *et al.* (2017) examined the impact of exposing Common carp to cadmium concentrations of 8.4 mg/L and lead concentrations of 6.2 mg/L for 15 days with a special focus on biochemical and hematological effects. The following parameters were checked: mean corpuscular volume (MCV) hemoglobin, hematocrit, lymphocytes, total protein, neutrophils, white blood cell (WBC), immunoglobulin M, glucose, red blood cells (RBC) and mean corpuscular hemoglobin concentration (MCHC). The results indicated significant reductions in WBC and MCHC levels ($P < 0.05$) with both metals. MCV decreased after Pb exposure ($P < 0.05$) but remained the same with Cd. MCH levels increased with both metals ($P < 0.05$) while other parameters remained similar to the control. IgM levels were elevated in Pb and Cd-exposed fish ($P < 0.05$). Gill histology showed lamellae fusion, vessel dilation, hyperemia and epithelial cell hyperplasia with unchanged muscle histology. The findings suggested that stress reactions are induced by low concentrations of heavy metals in the fish.

Abouelezz *et al.* (2020) examined the impact of feeding Nile tilapia fish with *Nigella sativa* on both their blood constituents and growth by observing three separate treatment groups: T_1 and T_2 were given *N. sativa* 1% and 2% respectively while The group that was used as the control received a basic diet. Each treatment group was further divided into 3 subgroups. Over 12 weeks, measurements of body weight, length and blood samples were taken fortnightly. The results have indicated a remarkable increase in both body weight and length increase in T_2. Hemoglobin levels are high in T_2 compared to the control. Serum albumin improved in T_1 and T_2 compared to control. Serum alkaline phosphatase increased notably in *N. sativa*-treated fish whereas serum glucose decreased as compared to normal. The inclusion of 1% and 2% dietary *N. sativa* had minor impacts on growth performance but the highest effects on constituents of blood in *O. niloticus*.

Latif and Faheem (2020) examined the outcomes of dietary supplementation with black seeds on the antioxidant activity and growth of *Labeo rohita*. Fingerlings were given diets comprising either 0.0%, 1.0%, or 2.5% *N. sativa* for twenty-eight days. The findings indicated that this fish fed on diets supplemented with black seed experienced improved growth rates. The black seed improved muscle protein and antioxidant activity. As a result, lipid peroxidation decreased and antioxidant enzyme levels increased in the gills, kidneys, liver and brain of rohu. The consumption of black seeds by Rohu fish resulted in a reduction of hepatic and nephric marker enzymes. The liver and kidney histo-architecture remained the same with the supplementation of black seed. The addition of 2.5% of this feed to the diet of rohu resulted in improved growth and reduced losses related to oxidative stress. The findings provided valuable insights for aquaculturists, nutritionists and researchers in formulating aquafeeds.

Abdel-Warith *et al.* (2020) conducted an experiment where *Clarias gariepinus* were subjected to varying concentrations of lead nitrate representing treatments of 0% (control), 20%, 40%, and 60% of the LC50 over 10 and 20 days. The accumulation of lead nitrate in gills increased after 10 days. After 20 days, these values further increased for the respective treatments. Liver accumulation of lead nitrate also increased with exposure duration and concentration, ranging from 3.32 ± 0.91 to 4.42 ± 0.78 mg/100 g of wet weight after 10 days. Skin and white muscles showed lower bioaccumulation initially but increased after 20 days. Hematological parameters like RBCs, Hb and Hct decreased significantly with increased $Pb(NO3)_2$ concentration while WBCs decreased as well. Liver enzyme activities of ALT and AST increased with both concentration and exposure time.

Yousefi *et al.* (2021) emphasized the importance of sustainable aquaculture in addressing global food production challenges. They investigated the impact of glyphosate on common carp fingerlings, a crucial cultured species, and explored the potential of dietary black seed supplementation (0.25%, 0.5%, 1%) to counteract toxicity. The study revealed that a 14-day glyphosate exposure induced oxidative stress, compromised antioxidant defenses and triggered immune depression in *Cyprinus carpio*. Fish supplemented with black seed-enriched diets for 60 days exhibited enhanced resilience to glyphosate, maintaining stable biochemical parameters, heightened immune defenses and increased antioxidant enzymes compared to control fish. The results suggested that incorporating black seed into the diet serves as a bio-remediation strategy which is very effective to mitigate the glyphosate toxicity in fish.

Latif *et al.* (2021) explored the potential protective impacts of *N. sativa* seeds against growth inhibition induced by diethyl-phthalate, changes in histo-biochemical parameters and oxidative stress in *Labeo rohita*. The experimental groups included Control, DEP alone, DEP combined with 1% *N. sativa* and DEP combined with 2.5% N. sativa. The experimental groups included Control, DEP alone, DEP combined with 1% N. sativa, and DEP combined with 2.5% *N. sativa*. The experimental groups included Control, DEP alone, DEP combined with 1% of *N. sativa* and DEP combined with 2.5% of *N. sativa*. It was incorporated into three diets at different levels and fish were treated with a sub-lethal concentration of DEP. The results revealed that DEP exposure led to decreased growth rates and disrupted protein contents in rohu, along with inhibition of antioxidant enzyme activities and increased lipid peroxidation. *N. sativa* supplementation mitigated these effects, promoting improved growth rates, restoring antioxidant enzyme activities and alleviating histopathological changes in the gills, kidney and liver. The study demonstrated the potential ameliorative role of seeds of *N. sativa* in protecting rohu against DEP toxicity.

Zulfahmi *et al.* (2021) examined the exposure impact of lead nitrate on the growth and hematology of female Milkfish fingerlings for 40 days. Lead nitrate was tested at four different concentrations: 0 mg/L, 42.64 mg/L, 63.97 mg/L and 85.29 mg/L. The results showed distinct changes in growth and hematology. The specific growth rate, feed conversion ratio, feed efficiency and weight gain of fish declined effectively. Lead nitrate exposure also reduced RBC count, hematocrit, hemoglobin levels and concentration of mean corpuscular hemoglobin while increasing MCV and MCH. Various malformations of erythrocytes were observed including deformation, swelling, doubling, binucleation, hemolysis, membrane laceration and vacuolation.

Hamed *et al.* (2022) experimented with the protective effects of cinnamon powder on tilapia (*Oreochromis niloticus*) fingerlings against prolonged lead (Pb) exposure. Fish were fed containing control, 10 g/kg CzP and 7.94 mg/L Pb diets for 60 days. Pb exposure adversely affected growth, WBC, RBC counts, hematocrit, hemoglobin levels and various biochemical parameters. The study suggested that lead caused toxicity in Nile tilapia.

Öz *et al.* (2024) investigated the effect of dietary *N. sativa* on the growth, hematology, biochemical profile and histopathology of Nile tilapia against cypermethrin toxicity. Fish were exposed to cypermethrin-contaminated water for 42 days. One percent *N. sativa* oil was supplemented into the diet to test its protective potential. The group receiving one percent black cumin oil exhibited the best growth while the group exposed to cypermethrin without black cumin oil showed the poorest growth. The study showed that adding black cumin to feed counteracted the adverse effects of cypermethrin, resulting in positive changes in growth, hematology, biochemistry and histopathology in Nile tilapia.

Materials and Methods

Heavy metals are excessively present in our environment and affect every single living organism all over the world. Fish are aquatic organisms that have direct exposure to heavy metals through water. The present research was performed to investigate the possible curative effects of *N. sativa* against lead.

Experimental Animals

Forty-eight fishes of normal weight and normal size of *C. catla* kept at the Department of Zoology, Wildlife and Fisheries in the University of Agriculture Faisalabad, (PARS) laboratory. They were purchased from Punjab Fish Hatchery, Satyana Road, Faisalabad. The fingerlings were acclimatized in a lab environment for one week.

Chemical Used:

N. sativa seed extract (10 g/kg) and lead (7 mg/L) were purchased from Sigma Aldrich, Germany.

Experimental design

Forty-eight fingerlings of C. *catla* were distributed into four treatments. T_0 received a basal diet and was considered as a control. T_1 was supplemented with lead (7 mg/L). T_2 was fed with *N. sativa* (10 g/kg). T_3 was fed with *N. sativa (*10 g/kg) and lead (7 mg/L). After 28 days of trial, fish were dissected and samples were collected from all treatments of fish for analysis.

Determination of growth performance and feed utilization

Growth performance was estimated through weekly gross weights of fingerlings from each experimental group. Feed utilization and growth performance were analyzed in terms of absolute weight gain (WG), specific growth rate (SGR), weight gain %, survival rate (%) as well as feed conversion ratio (FCR).

Weight gain (%)

The weight gain of fish was measured by following the formula

$$\text{Weight Gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Absolute weight gain (AWG)

The following formula was applied for the estimation of AWG in grams.

$$\text{Absolute weight gain(g)} = \text{Final weight(g)} - \text{Initial weight(g)}$$

Specific growth rate (SGR)

$$\text{SGR} = \frac{\text{Initial weight(g)} - \text{Final weight(g)}}{\text{Experimental duration (days)}} \times 100$$

Survival rate (%)

$$\text{Survival rate (\%)} = \frac{\text{Final number of fingerlings}}{\text{Initial weight of fingerlings}} \times 100$$

Feed conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Weight gain (g)}}$$

Hematological evaluation

The evaluation of hematological parameters was carried out with the help of an automated hematology analyzer.

Biochemical analysis

The levels of the liver enzymes ALT, ALP and AST were determined using ELISA kits.

Statistical analysis of data

Results of this study were displayed in the form of Mean ± SEM and it were computed by one-way ANOVA through SPSS software. The level of significance would be set at $p < 0.05$ (Inkielewicz-Stepniak *et al.*, 2012).

Results and discussion

The experiment was conducted to assess the effect of *N.sativa* on growth performance, liver enzymes and hematlogical parameters in *Catla catla*. Fourty eight fish were taken and allowed to acclimatize for a week and then randomly distributed in four treatments (T0,T1,T2 and T3) the total fish density was kept same as twelve fish per aquarium. All fish were fed on the same type of commercial food pellets twice a day at 10% body weight. *Catla catla* was exposed to *N.sativa* and lead. T0 was treated as control group while T1,T2 and T3 were exposed to *N.sativa*, lead and binary combination for four weeks. Growth performance was determined in terms of weight gain and length gain of fish on weekly basis while hematological parameters were estimated by Complete blood count test at the end of trial.

Assessment of growth performance

Growth rate was determined in terms of weight gain and total length gain on weekly basis. The assessment of growth in fish is a crucial aspect of aquaculture and biological research. This study aimed to evaluate the growth performance of fish through weekly measurements offering a comprehensive understanding of their development and informing best practices for sustainable fish farming and management.

Assessment of hematological parameters

The hematological parameters that is red blood cells, white blood cells, platelets, hematocrit and hemoglobin concentrations were assessed through CBS test. The measured hematology parameters were compared against known reference ranges for healthy fish. Hemoglobin and hematocrit levels within the normal range indicated adequate oxygen-carrying capacity and red blood cell production. The RBC and WBC counts provided insights into the fish overall health and immune status. Any deviations from the normal range were analyzed in relation to potential stress factors.

Assessment of liver enzymes

ALP, ALT and AST of liver were analyzed through biochemical analysis. Elevated levels of ALT and AST are indicative of liver cell damage or increased permeability of liver cell membranes. The observed enzyme activities were within the normal range for healthy fish suggested that the liver function was not adversely affected under the experimental conditions.

Table 4.1.Growth rate in different treatments of C.catla.

Groups	Weight gain (g)	Length gain (cm)
T0	27.12±1.16	10.47±0.88
T1	28.33±1.48	12.08±1.12
T2	20.90±1.18	6.85±1.16
T3	27.05±1.09	11.05±1.09

This table indicated that weight gain and length gain were minimum in lead exposed group as compared to other treatments.

Table 4.2 Comparison of different treatments on liver enzymes in C. catla

Liver enzymes	**T0**	**T1**	**T2**	**T3**
ALP	23.5±1	24.5±2	27.2±2.48	23.6 ±1
ALT	25.6±1.13	26.5±1.74	31.3±2.78	28.5±2.06
AST	53.2±1.75	54.1±2	57±2.70	53.3±1.74

This table indicated that there was an elevated levels of ALP, ALT and AST in lead exposed group as compared to other treatments

Table 3 : Hematological parameters of *C. catla* exposed to different treatments for four weeks.

Parameters	Treatments			
	T0	T1	T2	T3
Red blood cells	2.06±0.04	2.09±0.04	1.33±0.09	2.08±0.03
Hemoglobin	7.23± 0.01	8.2±0.14	5.12±0.05	7.35±0.004

Hematocrit	31.78±0.56	30.76±0.54	23.81±2.62	31.53±0.45
White blood cells	42.87±0.21	42.74±0.19	47.83±0.86	43±0.30
Platelets	314.21±0.35	313.76±0.20	290.93±0.39	310.93±0.23

This table indicating that the mean comparison of different treatments in which red blood cells, platelets , hemoglobin and hematocrit decreased while white blood cells increased in T2 as compared to other treatments.

Discussion

The experiment aimed to evaluate the effects of *Nigella sativa* (black seed) supplementation on growth performance, liver enzymes and hematological parameters in *Catla catla* exposed to lead. Medicinal plants such as *N. sativa* are increasingly used in aquaculture for their cost-effectiveness and dual roles as growth enhancers (Chen *et al*., 2016). Studies have demonstrated the benefits of adding medicinal herbs to fish diets with improvements noted in growth, immunity, digestion and disease resistance (Yousefi *et al*., 2021a).

In this study, growth performance was assessed weekly in terms of weight gain while hematological parameters were measured using a Complete Blood Count (CBC) test at the end of the trial. The results showed that fish fed *N. sativa*-supplemented diets exhibited a higher growth rate (28.33±1.48) compared to fish exposed to lead (20.90±1.18). This improvement is likely due to thymoquinone, the main growth-promoting component of *N. sativa*. These findings are consistent with previous studies that found improved growth in fish fed black seed supplements, such as in *Oreochromis niloticus* (Diab et al., 2008), *Cyprinus carpio* (Al-Dubakel et al., 2012), and *Anabas testudineus* (Khatun et al., 2015). Lead exposure significantly reduced growth rates aligning with findings from Hayat et al. (2007) who reported decreased weight gain in *Labeo rohita, Catla catla* and *Cirrhina mrigala* due to heavy metal exposure.

Nigella sativa supplementation also demonstrated hepatoprotective effects by reducing the levels of the liver enzyme AST in the treated fish (54.1±2) compared to the lead-exposed group (57±2.70). This reduction is attributed to the antioxidant properties of thymoquinone which protects the liver through mechanisms such as increasing antioxidant enzyme activity inhibiting lipid peroxidation and reducing lipogenesis in hepatocytes. Naeiji *et al*. (2013) similarly reported a decrease in AST levels with *Allium sativum* supplementation in *Cyprinus carpio*. The hepatoprotective effects of *N. sativa* are supported by Sarkar *et al*. (2021) who emphasized its high content of thymoquinone and other bioactive compounds such as alkaloids and vitamins.

Increased ALP enzyme levels were noted in the lead-exposed group (27.2±2.48) compared to the *N. sativa* treated groups (24.5±2 and 23.6±1). Elevated ALP levels indicated liver damage and stress as confirmed by studies on *Cirrhinus mrigala* exposed to cypermethrin (Prashanth and Neelagund, 2008) and *Labeo rohita* exposed to cypermethrin (Tiwari et al., 2012). Similarly, ALT enzyme levels were higher in the lead-exposed group (31.3±2.78) compared to *N. sativa* treated groups (26.5±1.74 and 28.5±2.06) indicating liver toxicity. These results are aligned with findings by Faheem *et al*. (2019) and Poopal *et al*. (2017) who noted elevated ALT levels in fish under toxic stress.

Hematological parameters revealed that lead exposure significantly reduced RBC levels (1.33±0.09) compared to the control group (2.06±0.04). Lead interferes with enzymes crucial for heme synthesis such as δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase which disrupted RBC production. Similar findings were reported by Mekkawy *et al*. (2011) and Hedayati and Ghaffari (2013) in *Oreochromis niloticus* and freshwater fish exposed to heavy metals. WBC levels increased in the lead-exposed group (47.83±0.86) compared to the control (42.87±0.21). This may be attributed due to stress-induced cortisol release and enhancing immune responses. This is supported by studies from Alak *et al*. (2018) and Rauta *et al*. (2022), who reported increased WBC counts in fish under stress or disease conditions.

Hematocrit levels also decreased in the lead-exposed group (23.81±2.62) compared to the control (31.78±0.56) which is a common effect of heavy metal toxicity on erythropoiesis leading to reduced RBC production and increased destruction. These results are consistent with Mekkawy *et al.* (2011) and Hedayati and Ghaffari (2013), who observed similar hematocrit reductions in fish exposed to cadmium and nickel. Hemoglobin levels were similarly reduced in the lead-exposed group (5.12±0.05) compared to the *N. sativa* treated group (8.2±0.14). It may be attributed due to lead which disrupts bone marrow function and inhibits hemoglobin synthesis. This is in line with the findings of Mekkawy et al. (2011).

Lead exposure significantly reduced platelet levels (290±93) compared to the control group (314.21±0.25). It may be attributed due to lead interference with megakaryocyte maturation and platelet production. Similar reductions in platelet counts were reported by Ahmed *et al*. (2022) and Qadir *et al*. (2021) in fish exposed to heavy metals. In conclusion, lead exposure adversely affects growth, hematological parameters and liver function in *Catla catla*. However, dietary supplementation with *Nigella sativa* at 10 g/kg mitigates these effects of improving growth performance, stabilizing hematological parameters and reducing liver enzyme levels. While the study demonstrated the short-term protective effects of *N. sativa* further research is required to elucidate the underlying biochemical mechanisms.

References

Abdel-Warith, A.-W.A., E.-S.M. Younis, N.A. Al-Asgah, A.M. Rady and H.Y. Allam. 2020. Bioaccumulation of lead nitrate in tissues and its effects on hematological and biochemical parameters of *Clarias gariepinus*. Saudi J. Biol. Sci. 27:840-845.

ABOUELEZZ, K., M. EL-KELLAWY and D. YA. 2020. Effect of Nigella sativa on growth performance and some blood constituents of Nile Tilapia (*Oreochromis Niloticus*). Assiut Vet. Med. J. 66:121-135.

Ahmad, I., J. Tripathi, S. Manik, L. Umar and J. Rabia. 2013. Preliminary phytochemical studies of the miracle herb of the century, *Nigella sativa* L.(Black Seed). Indo Am. J. Pharm. Res. 3:3000-3007.

Al-Busaidi, M., P. Yesudhason, S. Al-Mughairi, W. Al-Rahbi, K. Al-Harthy, N. Al-Mazrooei and S. Al-Habsi. 2011. Toxic metals in commercial marine fish in Oman with reference to national and international standards. Chemosphere 85:67-73.

Aloo, P., H. Charo-Karisa, J. Munguti and B. Nyonje. 2017. A review on the potential of aquaculture development in Kenya for poverty alleviation and food security. AFJAND. 17:11832-11847.

Brraich, O.S. and L. Kaur. 2022. Comparative studies on age and growth patterns of cultivated and wild *Catla catla* (Hamilton). IJEB. 60:144-149.

Chen, X.J., J. Wang and Q. Wang. 2016. Research and application of Chinese herbs in aquaculture of China. SSRG Int. J. Med. Sci. 3:8-13.

Cojocaru, A.L., Y. Liu, M.D. Smith, W. Akpalu, C. Chávez, M.M. Dey, J. Dresdner, V. Kahui, R.B. Pincinato and N. Tran. 2022. The "seafood" system: Aquatic foods, food security, and the Global South. REEP. 16:306-326.

Gabriel, N.N., J. Qiang, X.Y. Ma, J. He, P. Xu and K. Liu. 2015. Dietary Aloe vera improves plasma lipid profile, antioxidant, and hepatoprotective enzyme activities in GIFT-tilapia (*Oreochromis niloticus*) after Streptococcus iniae challenge. Fish Physiol. Biochem. 41:1321-1332.

Garai, P., P. Banerjee, P. Mondal and N. Saha. 2021. Effect of heavy metals on fishes: Toxicity and bioaccumulation. J. Clin. Toxicol. S 18

Hamed, H.S., S.M. Ismal and M. Abdel-Tawwab. 2022. Modulatory effects of dietary cinnamon (*Cinnamomum zeylanicum*) against waterborne lead toxicity in Nile tilapia fingerlings: Growth performance, haemato-biochemical, innate immunity, and hepatic antioxidant indices. Aquac. Rep. 25:101190.

Harikrishnan, R., G. Devi, H. Van Doan, K. Gatphayak, C. Balasundaram, E. El-Haroun and M. Soltani. 2022. Immunomulation effect of alginic acid and chitooligosaccharides in silver carp (*Hypophthalmichthys molitrix*). Fish Shellfish Immunol. 128:592-603.

Inkielewicz-Stepniak, I., M.W. Radomski and M. Wozniak. 2012. Fisetin prevents fluoride-and dexamethasone-induced oxidative damage in osteoblast and hippocampal cells. FCT. 50:583-589.

Ishaque, A., S. Ishaque, A. Arif and H. Abbas. 2020. Toxic effects of lead on fish and human. Biol. Clin. Sci. Res. J. 2020

Khalesi, M., Z. Abedi, S. Behrouzi and S.K. Eskandari. 2017. Haematological, blood biochemical and histopathological effects of sublethal cadmium and lead concentrations in common carp. Bulg. J. Vet. Med. 20:141-150.

Kim, J.-H. and J.-C. Kang. 2015. The lead accumulation and hematological findings in juvenile rock fish Sebastes schlegelii exposed to the dietary lead (II) concentrations. Ecotoxicol. Environ. Saf. 115:33-39.

Latif, M. and M. Faheem. 2020. Asmatullah; Hoseinifar, SH; Van Doan, H. Dietary Black Seed Effects on Growth Performance, Proximate Composition, Antioxidant and Histo-Biochemical Parameters of a Culturable Fish, Rohu (*Labeo rohita*). J.Anim. 2021, 11, 48.

Latif, M., M. Faheem, S.H. Hoseinifar and H. Van Doan. 2021. Protective efficacy of *Nigella sativa* seeds against diethyl phthalate induced growth retardation, oxidative stress and histo-biochemical damages in *Labeo rohita*. Aquac. 533:736065.

Öz, M., E. Üstüner and F. Bölükbaş. 2024. Effects of dietary black cumin (*Nigella sativa* L.) oil on growth performance, hemato-biochemical and histopathology of cypermethrin-intoxicated Nile tilapia (*Oreochromis niloticus*). JWAS. 55:273-288.

Reverter, M., N. Bontemps, D. Lecchini, B. Banaigs and P. Sasal. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquac. 433:50-61.

Schuhladen, K., J.A. Roether and A.R. Boccaccini. 2019. Bioactive glasses meet phytotherapeutics: The potential of natural herbal medicines to extend the functionality of bioactive glasses. Biomater. 217:119288.

Shahid, F., Z. Farooqui, S. Rizwan, S. Abidi, I. Parwez and F. Khan. 2017. Oral administration of *Nigella sativa* oil ameliorates the effect of cisplatin on brush border membrane enzymes, carbohydrate metabolism and antioxidant system in rat intestine. Toxicol. Pathol. 69:299-306.

Shahjahan, M., K. Taslima, M.S. Rahman, M. Al-Emran, S.I. Alam and C. Faggio. 2022. Effects of heavy metals on fish physiology–a review. Chemosphere 300:134519.

Sharma, R. and R. Jindal. 2020. Assessment of cypermethrin induced hepatic toxicity in *Catla catla*: A multiple biomarker approach. Environ. Res.J. 184:109359.

Woo, C.C., A.P. Kumar, G. Sethi and K.H.B. Tan. 2012. Thymoquinone: potential cure for inflammatory disorders and cancer. Biochem. Pharmacol. 83:443-451.

Yousefi, S.R., H.A. Alshamsi, O. Amiri and M. Salavati-Niasari. 2021. Synthesis, characterization and application of Co/Co3O4 nanocomposites as an effective photocatalyst for discoloration of organic dye contaminants in wastewater and antibacterial properties. J. Mol. Liq. 337:116405.

Zulfahmi, I., A. Rahmi, M. Muliari, Y. Akmal, E. Paujiah, K.A. Sumon and M.M. Rahman. 2021. Exposure to lead nitrate alters growth and haematological parameters of milkfish (*Chanos chanos*). BECT. 107:860-867.