IRSTI 34.47.51 https://doi.org/10.26577/IJBCh2024v17i1-a7

J. Iqra, A. Shabbir, A. Hasnain, A. Iqra,
S. Muqadas, A. Sajid, K. Sheraz, Sh. Khurram*

University of Okara, Okara, Pakistan
*e-mail: dr.khurram@uo.edu.pk

(Received 3 May 2024; received in revised form 12 May 2024; Accepted 28 May 2024)

The potential ameliorative effects of *Moringa oleifera* in emamectin benzoate induced toxicity in freshwater *Labeo rohita* fish

Abstract. Emamectin benzoate pesticide (EMB) is life-threatening chemical causing harmful effects on aquatic organisms specially fish species. Current study was aimed to understand the ameliorative role of *Moringa oleifera* against the toxic effects of emamectin benzoate on hematological, biochemical and changes in the soft tissues of *Labeo rohita*. Four groups were made. First group without chemicals is the control group, while 2nd, 3rd and 4th groups were exposed to 3.64µg/L, 7.28µg/L and 14.56µg/L EMB respectively for 30 days. Results revealed that the number of RBCs, HGB, HCT, MCV, MCH, MCHC, and WBCs were decreased while lymphocytes, monocytes, eosinophils and platelets were increased significantly. Biochemical parameters of fish including serum cholesterol, triglycerides, LDL, VLDL, albumin, globulin, blood glucose, ALT, AST, TSH, urea, creatinine, and blood urea nitrogen were significantly increased while HDL, total protein, T3 and T4 were decreased. Histological changes revealed that EMB causes degenerative effects in soft tissues of exposed fish. After using *Moringa oleifera*, all the effects were reversed and the fish was moved towards recovery. The overall results of the study showed that emamectin benzoate can alter the histological, hematological, and biochemical processes of *Labeo rohita,* thus toxic to aquatic organisms.
Key words: pesticides, recovery responses, hepatotoxicity, nephrotoxicity, hemotoxicity, fish.

Introduction

Pesticides are natural compounds that are poisonous to pests and also life threatening to aquatic animals because they enter the water bodies through different processes such as drainage and droplets [1]. Emamectin benzoate is a member of the avermectins family and it is separated from the bacteria *Streptomyces avermitilis*. It is extensively used as a germicide and insecticide in the agricultural field to kill different pests, and is also used in the aquatic environment [2]. EMB is toxic to nerve cells and it remains deposited in the soil [3] and moves to marine and freshwater through different processes such as decaying feed and feces. It is less toxic to mammals, birds and some useful arthropods [4]. EMB is lethal to humans as it depressed the activity of different body organs and induces toxic effects on the kidney [5], liver [6], nervous system, heart [7].

EMB toxicity has side effects on different animals such as in rats it can cause problems in the eyes and nervous system, slow down the phagocytic process, and functioning of macrophages [8], cause teratogenic effects [9]. EMB is also lethal to mice as it changes the histopathology of the liver, kidney, and brain [10] and it also causes reproductive toxicity [11].

EMB induces toxic effects on fish in many ways such as it affects the embryo, causing malformation, and slowing down the hatching process [7]. It is lethal to tissues, alters the heart working, decay epithelial tubes in the kidney and diminishes its working, blocks the glomerulus, and proximal and distal tubules become swollen [4, 12, 13]. It is also toxic to the liver [14], induces hyperglycemia, obesity, diabetes, and enhance the level of glucose [15].

Fish is a very sensitive and dominant organism in aquatic environments used for the detection of toxicity and also a very good bio-indicator in the research field to study the harmful and beneficial effects of chemicals in water bodies [16]. *Labeo (L.) rohita* (Rohu) is a freshwater fish and it is the most common and favorable fish in South Asian countries including Pakistan, India, Bangladesh and Nepal. It is adjusted to laboratory conditions easily. Many studies have been done to investigate the changes

in the hematology of *Labeo rohita* treated with emamectin benzoate [17].

To the best of our knowledge, it is first investigation on the effects of Moringa oleifera against the toxic effects in freshwater teleost. Therefore, present study aimed to check the ameliorative effects of Moringa oleifera against the toxic effects of emamectin benzoate in freshwater fish L. rohita using different biomarkers including, hematology, lipid profile, thyroid functioning, liver and kidney functioning and histological changes.

Materials and methods

Experimental animal. The experimental fish used in the present study is *Labeo rohita* purchased from the Head Balloki fish hatchery, Kasur, Pakistan, and transported to the aquaculture lab of the University of Okara. No mortality was observed during transportation. Fish were acclimatized in a large glass tank for seven days. During the whole experiment, after every 48 hours, the excreta of fish and the remaining food were removed from the aquarium, by using a recirculation aerated system along with a water renewal system. Fish were kept at a set temperature of 29.5±27°C, pH of 7.46±0.26 of water, and oxygen concentration of 7.25±0.23 mg/L.

Experimental chemical. Emamectin benzoate was purchased from Punjab agrochemical trader, Okara, Pakistan. *Moringa oleifera* powder was purchased from an herbal store. Appropriate concentration of EMB was dissolved in distilled water for stock solution.

Experimental design. The present experiment was designed for 30 days. During the experiment fish were held in glass aquariums that contains 40 liters of water. Domestic feed was used after 48 hours during the duration of the experiment. Depending upon the concentration of emamectin benzoate four types of treatment groups were designed. Each group contained twenty fish. The first group without chemicals is the control group. 2nd, 3rd and 4th groups were exposed to emamectin benzoate concentrations of 3.64 µg/L, 7.28 µg/L and 14.56 µg/L, respectively. The approval No. UO/ERC/2023/46A from September 14, 2023 was achieved from the ethical research committee of the University of Okara, Pakistan.

Hematological analysis. At day 30, fish (n=12) from each glass aquarium were removed for blood collection. Blood was extracted through BD syringe from caudal vein and stored in EDTA tubes for hematology and without EDTA for serum biochemical analysis. Hematological parameters, such as RBC, HGB, HCT, MCV, MCH, MCHC, WBC, PDW, RDW-SD, Neutrophils, lymphocytes, monocytes, eosinophils, RDW-CD, PLT, MPV, and PCT were analyzed by automatic hematological analyzer SMT-50 (Chengdu Seamaty Technology Co., Ltd, China).

Biochemical analysis. Blood sample were left for coagulatory process for two hours at room temperature. Biochemical analysis was performed in Lifeline laboratory and diagnostic center in Lahore, Pakistan. Biochemical analysis of different parameters, such as cholesterol, triglycerides, HDL, LDL, VLDL, AST, ALT, total protein, albumin, globulin, blood glucose, BUN, urea, creatinine, T3, T4 and TSH hormones was performed in the laboratory conditions.

Histological studies. After blood collection, fish were dissected. All organs (gills, liver and kidney) were removed and fixed in a solution containing ethanol, formalin, and acetic acid (ALFAC), then stored in 70% ethanol. Tissues were embedded in paraffin, sectioned (5 µm), and the slides stained with hematoxylin and eosin. The sections were examined under light microscope using a digital camera.

Recovery responses. At day 30, five fish from group 4th (14.56µg/L) removed and transferred to a chemical free freshwater aquarium. For recovery responses, fish were given moringa leaf powder mixed with feed according to body weight for seven days. After seven days, fish removed for above mentioned assays.

Statistical analysis. The completely randomized experimental design results are presented as mean ± standard deviation and one-way analysis of variance ANOVA. Version of ANOVA that was used in analysis is ordinary one-way ANOVA from GraphPad prism (version 9.3.1).

Results and discussion

The results of many studies show that teratological disorders, genotoxic effects, embryonic toxicity and histological damages can be caused by carbamate, organophosphate, and organochlorine pesticides [18, 19]. In our experiment, emamectin benzoate was used to test the toxicity based on changes in blood parameters, biochemical parameters, and histology of fish *Labeo rohita.*

Hematological analysis. Results of the hematological parameters of *L. rohita* are shown in Table 1.

Table 1 – Showing alterations in hematological parameters of *L. rohita* in control group, emamectin benzoate exposed groups and *Moringa oleifera* exposed group. Data are represented as mean ± SD. *= $p< 0.05$ level of significance, NS=non-significant, Mo=*Moringa oleifera*

Parameters	Control	Low	Med	High	Mo
HGB (g/dl)	5.63±0.25	4.53±0.45 ^{NS}	3.70±0.55*	2.83±0.75*	4.66±0.72*
WBC (x10^3/µL)	15.80±0.55	14.20±0.72*	12.13±0.56*	10.23±0.60*	13.20±0.52*
RBC (x10^6/ µL)	1.17±0.02	1.05±0.04^{N}S	1.00±0.04*	0.91±0.07*	1.04±0.04*
HCT (%)	15.00±0.55	12.70±0.72*	11.90±0.62*	10.80±0.81*	11.27±0.7^{6N}S
MCV (FL)	141.9±1.17	138.7±1.10*	134.8±1.22*	131.0±1.48*	136.4±1.22*
MCH (pg)	47.57±0.60	46.07±0.76*	42.97±0.66*	41.20±0.60*	43.70±0.62*
MCHC (g/dl)	113.0±2.00	98.33±5.03*	76.67±3.05*	65.67±3.05*	50.67±4.50*
RDW-CD (%)	23.80±1.05	28.53±0.60*	36.50±1.70*	43.70±0.98*	42.27±0.86^{NS}
RDW-SD (%)	32.47±3.90	24.40±3.90*	17.67±2.07*	9.50±0.60*	10.50±0.85^{NS}
PLT(x10^3/µL)	219.0±52.85	381.7±45.08*	535.3±38.19*	679.0±38.74*	647.7±37.63^{NS}
MPV (FL)	5.36±0.55	7.30±0.55*	8.56±0.6506*	9.40±0.65*	8.93±0.70^{NS}
PDW (%)	15.50±1.86	9.40±1.45*	5.66±0.85*	2.80±1.15*	3.46±0.49^{NS}
PCT (%)	0.10±0.02	0.24±0.03*	0.46±0.06*	0.62±0.05*	0.54±0.06^{NS}
Neutrophils %	69.67±5.50	81.67±4.50*	89.33±4.50*	97.33±5.50*	87.33±3.05 ^{NS}
Lymphocytes (%)	25.33±7.02	54.33±7.50*	74.33±8.02*	87.67±8.50*	84.63±3.51^{NS}
Monocytes (%)	2.033±0.55	4.10±0.55*	5.96±0.45*	8.96±0.65*	7.86±0.77^{NS}
Eosinophils (%)	2.26±0.25	2.70±0.20^{NS}	3.26±0.25*	4.16±0.15*	3.80±0.26^{NS}

RBCs, HGB, HCT, MCV, MCH, MCHC, WBCs, PDW and RDW-SD were decreased significantly in the treated group as compared to the control group. Some other parameters, like neutrophils, lymphocytes, monocytes, eosinophils, RDW-CD, PLT, MPV and PCT increased significantly in experimental groups compared to the control group**.** Use of moringa leaf powder shows that some parameters including RBC, HGB, and WBC, MCV, MCH, and MCHC were significantly increased.

Biochemical analysis. Results of the effects of emamectin benzoate and recovery responses after moringa leaf powder on the biochemical parameters and thyroid hormone of *L. rohita* are presented on Figures 1, 2, and 3. Assessment of biochemical parameters reveals information on the functioning of important organ systems such as the immune system, hematopoietic system, kidneys, and liver. These are commonly employed in toxicological investigations to identify and assess cell damage [28]. According to current study a significant increase was observed in lipid profile such as triglycerides, cholesterol, low density lipoproteins and very low-density lipoproteins except high density lipoproteins after exposure to EMB (Figure 1).

Results indicate that all the biochemical parameters (except high density lipoproteins, serum total proteins, T3 and T4) showed a significant increase in experimental groups than in control group. Blood sugar level undergoes a significant increase in EMB exposed group. The level of thyroid hormones T3 and T4 significant decrease but the level of TSH increased in the treated group as compared to the control group. After the recovery by using moringa leaf powder, the levels of cholesterol, triglyceride, LDL, urea, creatinine, blood glucose, ALT, AST, serum total proteins and albumin showed significant recovery responses. Remaining parameters were also restored to some extent.

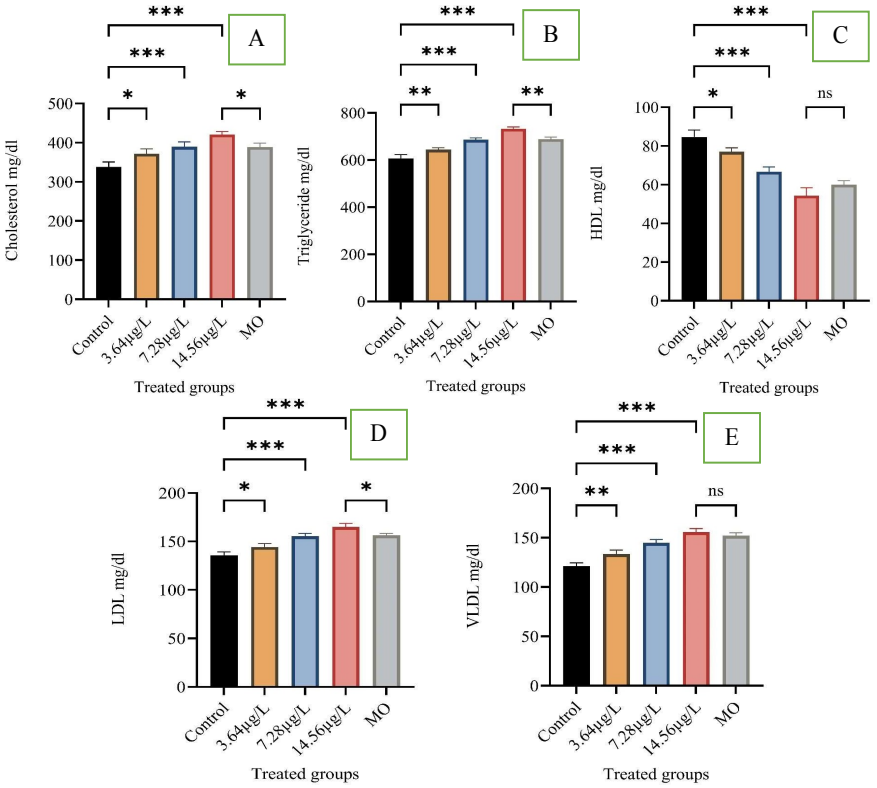

Figure 1 – Graphical representation shows the variation in (A) cholesterol, (B) triglycerides, (C) HDL, (D) LDL and (E) VLDL of *L. rohita* after exposure of emamectin benzoate and *Moringa oleifera*.

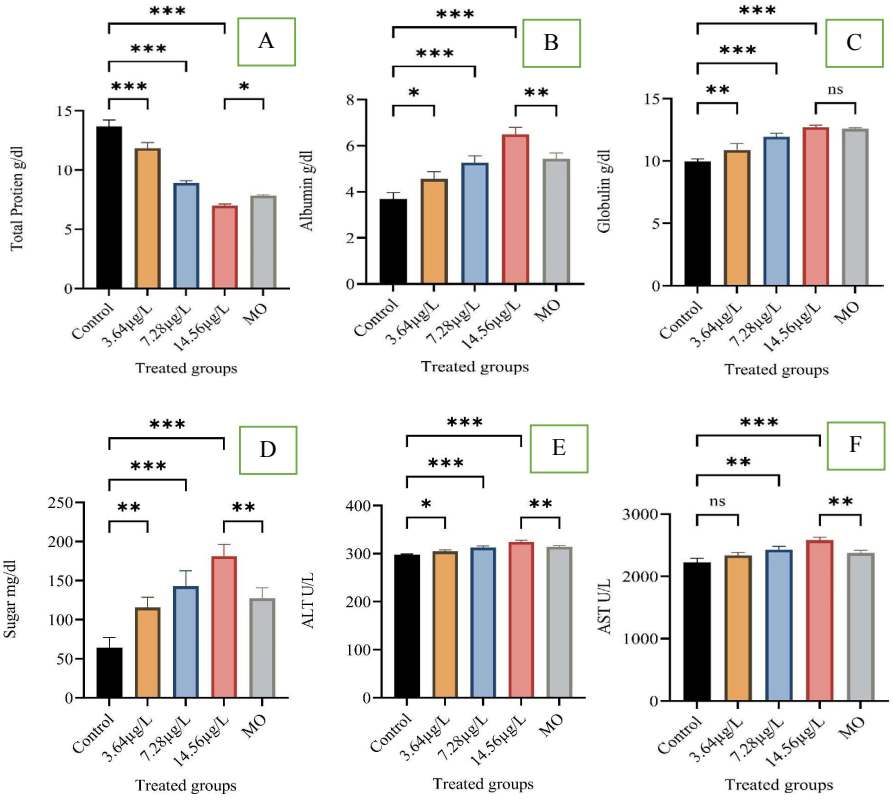

Figure 2 – Graphical representation shows the variation in (A) total protein, (B) albumin, (C) globulin, (D) blood glucose, (E) ALT and (F) AST of *L. rohita* after exposure of emamectin benzoate and *Moringa oleifera*

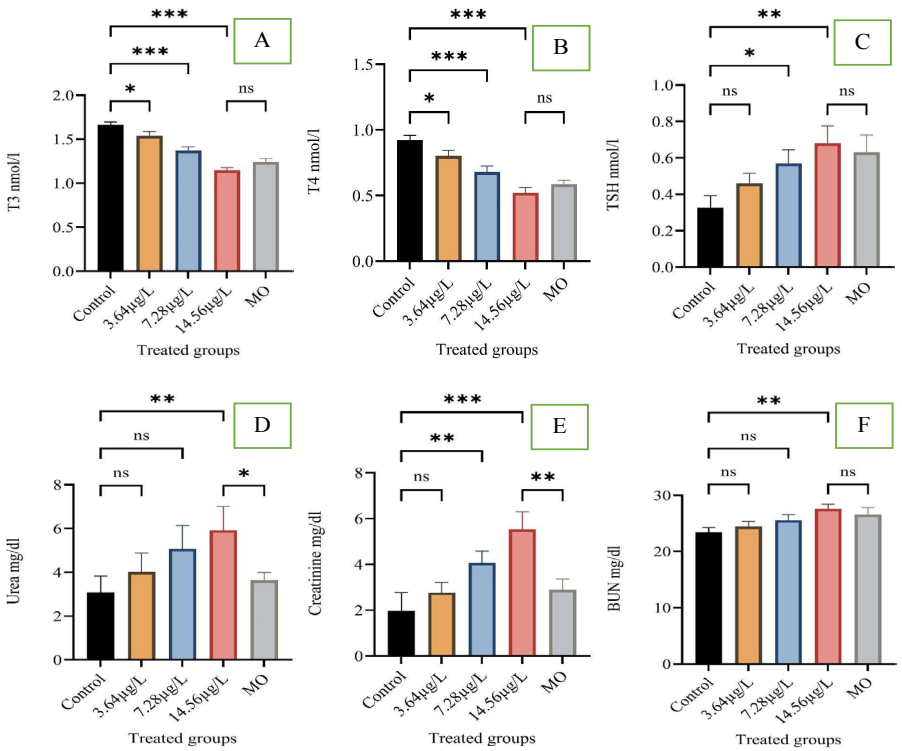

Figure 3 – Graphical representation shows the variation in (A) T3, (B) T4, (C) TSH, (D) urea, (E) creatinine and (F) BUN (blood urea nitrogen) of *L. rohita* after exposure of emamectin benzoate and *Moringa oleifera*.

Hematological changes caused by exposure to xenobiotics serve as a good indication of the physiological condition in fish [20], birds [21] and other vertebrates [22].

The current study revealed that RBC, HGB, MCH, MCV, and MCHC were significant decreases in experimental groups in comparison to the control group. Toxicity of EMB causes direct injurious effects on the RBC cells, destruction of red blood cells, shrinkage of RBC, erythrocytic lysis, and inhibition of hematopoiesis, which causes the reduction of red blood cells. Similar findings were observed by Kumar et al. [17] and Alghamdi [23].

Results showed that WBCs significantly decreases in blood due to inhibition of the maturation process and immunity suppressions caused by toxicity after being exposed to EMB but significant increase in the lymphocytes, neutrophils, monocytes and eosinophils was indications of chronic infestations. Significant increase in platelets count observed due to infections caused by EMB exposure. These observations are supported by Kumar et al. [17]. After the recovery by using moringa leaf powder showed that RBCs and HGB were significant increases in treated group. Similar findings were observed by Suzana et al. [24]. Other parameters such as WBC, MCV, MCH and MCHC were significant increased which are supported by previously published literature [25-27].

Histology. Histological studies of *Labeo rohita* after exposure to EMB showed that liver tissues undergo different changes such as pyknotic nuclei, cluster nuclei formation, and necrosis in exposed fish (Figure 4).

Gills tissues showed different changes like curved gill lamellae, damaged epithelial cell, bone cell deformities, edema and fused gill lamellae in exposed fish (Figure 5).

Kidney tissues demonstrated some alterations such as sinusoidal spaces, cluster nuclei formation, melanomacrophage, damaged epithelial cells, and elongated tubules in exposed fish (Figure 6).

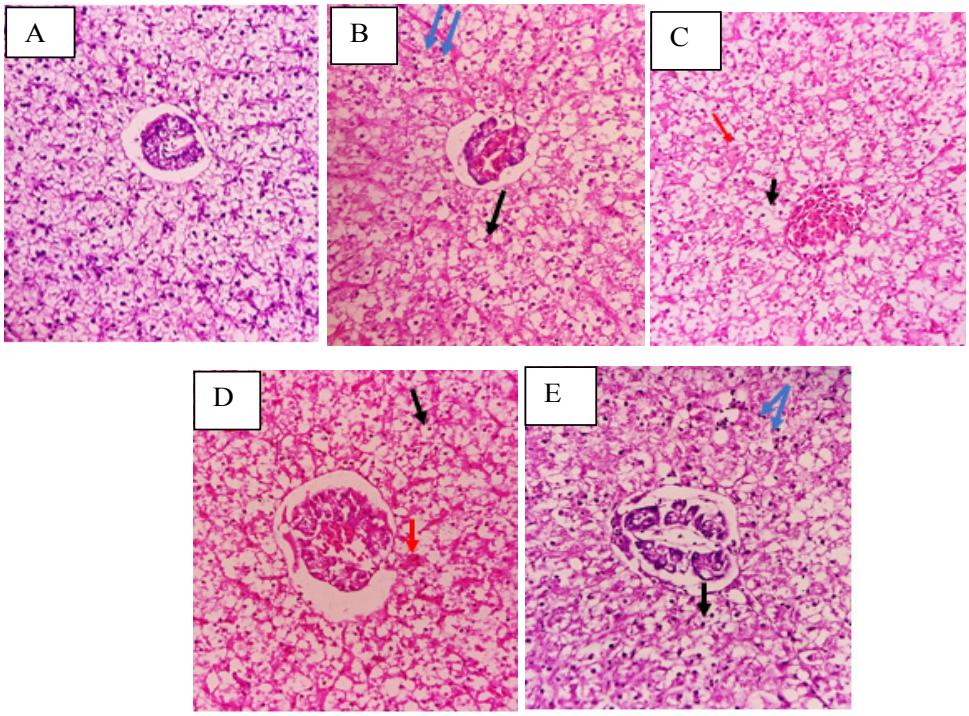

Figure 4 – Histology of liver tissues of *Labeo rohita* at 40X. (A) control group, (B) low dose group, (C) medium dose group, (D) high dose group and (E) recovery group. Black arrows show pyknotic nuclei, blue arrows show cluster nuclei formation, red arrows show necrosis.

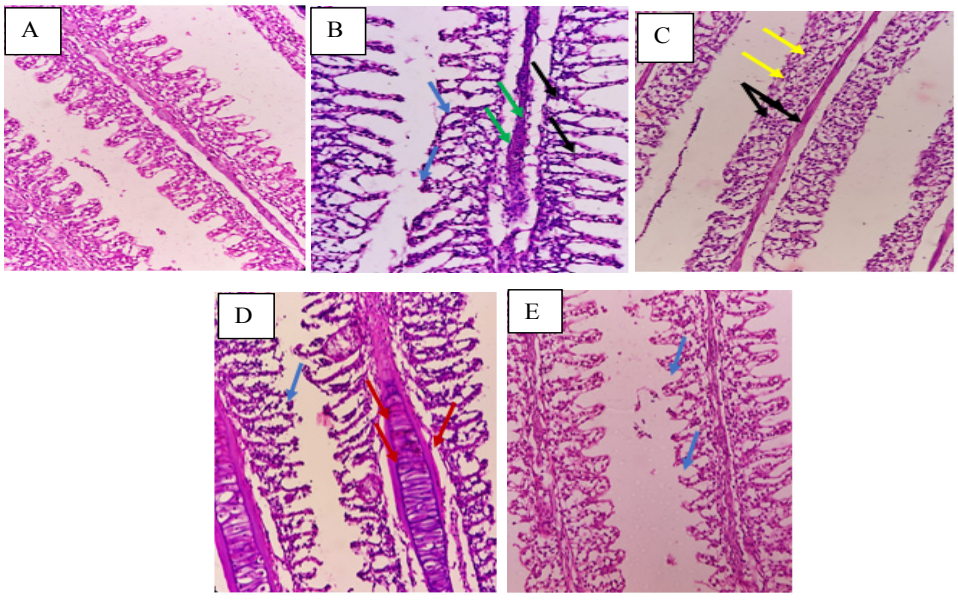

Figure 5 – Histology of Gill tissues of *Labeo rohita* at 40X. (A) control group, (B) low dose group, (C) medium dose group, (D) high dose group and (E) recovery group. Blue arrows show curved gill lamellae, black arrows show damaged epithelial cell, green arrows show bone cell deformities, red arrows show oedema, yellow arrows show fused gill lamellae.

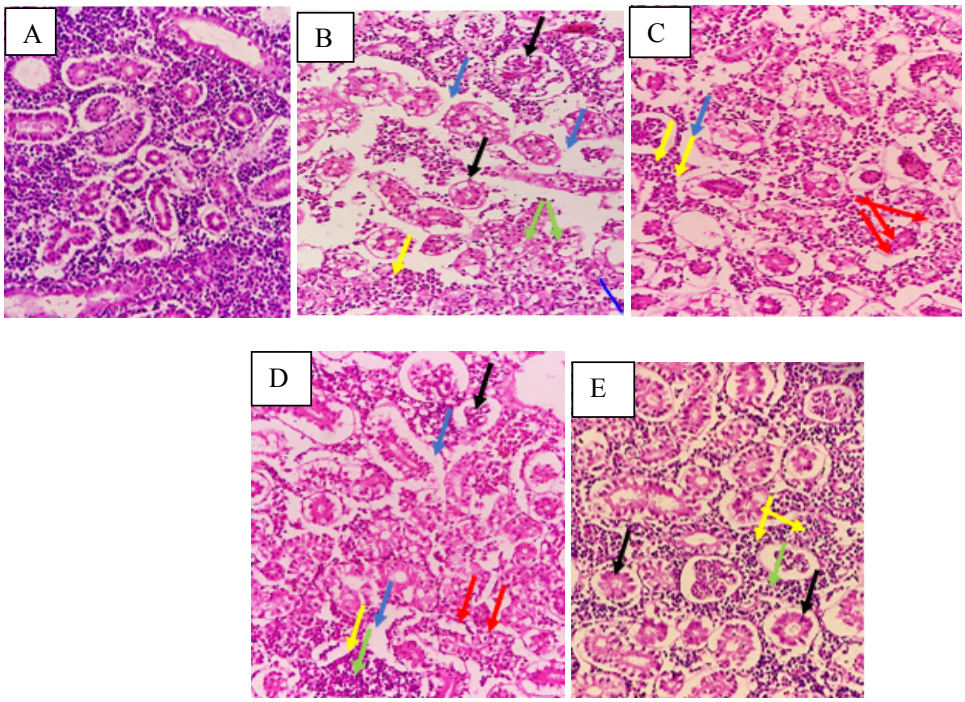

Figure 6 – Histology of kidney tissues of *Labeo rohita* at 40X. (A) control group, (B) low dose group, (C) medium dose group, (D) high dose group and (E) recovery group. Blue arrows show sinusoidal spaces, yellow arrows show cluster nuclei formation, black arrows show melanomacrophage, green arrows show damaged epithelial cell, red arrows show elongated tubules

After recovery period, using moringa leaf powder, liver, kidney and gill tissues showed recovery to some extent from these damages.

It is because of liver damages (Figure 4) and malfunctioning of liver increase the level in blood. Cholesterol plays role in cell membrane and steroid hormone which act as major indicator to check the stress caused by any chemical. Increased cholesterol level showed the toxicity caused by EMB. Same observations noted by Lee, et al. [29]. ALT and AST showed significance increase in exposed groups in comparison to control. These enzymes present in liver cells help in conversion of amino acid into alpha-keto acids, any abnormality in hepatocytes lead to an increase the level of these enzymes in blood which show toxicity and necrosis in liver. Similar results observed by Ogueji, et al. [2], [17] and Cheng, et al. [3]. Higher levels of albumin and globulin may be due to increase excretory and synthetic functions of liver while decrease the level of serum total proteins in experimental animals due to decrease protein synthesis. Protein play the role in removal, transport and distribution of toxic substances [30]. Blood glucose level increased in the experimental animals exposed to EMB in comparison to control group. Due to stress caused by toxic chemical, increased gluconeogenesis in liver and disturbance in the metabolism of protein and lipid in order to compensate the increased energy demand. Similar results were observed by Alghamdi [23] and Kumar, et al. [17]. A significant increase was observed in TSH level while decreased T3 and T4 levels in treated groups as compared with control group. Thyroid hormone plays important role in growth, differentiation and maintenance of metabolic process. Any abnormality in the thyroid hormones show that damages in the structure and functions of gland take place which leads to decrease the level of these two hormones in blood. Pituitary gland send signal for more TSH to fulfill the energy demands that is why TSH level increase in the blood. Our findings correspond with those of Azoz, et al. [31]. Increased levels of urea, blood urea nitrogen and creatinine in blood indicated that changes and malfunctioning of kidney due to toxic effects of EMB. Due to kidney damage (figure 6), it is unable to filter the urea, creatinine and BUN in blood and its level increase in blood [32, 33]. By using the moringa leaf powder, recovery responses showed that cholesterol, triglyceride, LDL, urea, creatinine, ALT, AST, serum proteins and blood

glucose levels were significantly restored to some extent. Our observations are similar to El-Kassas, et al. [34] and El-bakry, et al. [35].

Toxin-induced organ damage and injury level may be assessed by histological alterations, which have shown to be highly valuable assessments [36]. As the primary organ for detoxification, the liver serves as a useful biomarker of chemical toxicity. Histological studies of *Labeo rohita* after exposure to EMB showed that liver tissues undergo various changes such as pyknotic nuclei, cluster nuclei formation, and necrosis. Khaldoun-Oularbi, et al. [5] reported similar changes in rats. The gills are vital organs that play an important part in metabolic processes, act as a barrier, and regulate osmoregulation and excretion in addition to being the principal targeted tissues for respiration. Pesticides primarily enter the body through the gills [20, 37]. Fish exposed to EMB showed different changes in gill tissues such as curved gill lamellae, damaged epithelial cell, bone cell deformities, edema and fused gill lamellae. The kidneys play a crucial role in the body's waste removal process. Toxin concentrations affect the structural alterations that the kidneys go through in response to toxin elimination [16]. Fish exposed to EMB showed different changes in kidney tissues such as sinusoidal spaces, cluster nuclei formation, melanomacrophage, damaged epithelial cells, and elongated tubules. Kumar, et al. [17] reported similar damage effects in *Labeo rohita*. After exposure to moringa leaf powder, liver, kidney and gill tissues showed good recovery responses from these damages. Moringa leaf powder is very effective for restoration of organ toxicity [38].

Conclusion

Current results indicated that emamectin benzoate (EMB) have toxic effects on freshwater fish *Labeo rohita* at low concentrations. EMB undergoes alterations in hematological and biochemical parameters in experimental groups in comparison to control group. Changes in Lipid profile, serum proteins, thyroid hormones and urea levels are the evidence of emamectin benzoate toxicity. It also cases degenerative and damaging effects in gills, liver and kidney of exposed fish. Results indicated that *Moringa oleifera* is an excellent object for restoration of toxicity caused by EMB. The authors would like to acknowledge chairperson Department of Zoology, University of Okara, Pakistan. This research did not receive any kind of financial support and part of postgraduate research work.

References

1. Ullah S. et al. (2018) Cypermethrin induced toxicities in fish and adverse health outcomes: its prevention and control measure adaptation, J Environ Manage., 206, 863-871.

2. Ogueji E. et al. (2023) Histological and hematological changes to Clarias gariepinus juveniles exposed to acute doses of Emamectin benzoate in a static bioassay, Ecotoxicol., pp. 1-14.

3. Cheng B. et al. (2020) The chronic toxicity of emamectin benzoate to three marine benthic species using microcosms, Ecotoxicol Environ Saf., 194, 110452.

4. Raja R.A., Patil P.K., Avunje S., Aravind R.P., Alavandi S.V., Vijayan K.K. (2020) Biosafety, withdrawal and efficacy of anti-parasitic drug emamectin benzoate in Asian Seabass (*Lates calcarifer*) *Aquaculture,* 525, 735335.

5. Khaldoun-Oularbi H., Allorge D., Richeval C., Lhermitte M., Djenas N. (2015) Emamectin benzoate (Proclaim®) mediates biochemical changes and histopathological damage in the kidney of male Wistar rats (Rattus norvegicus), Toxicol. Anal. Clin., 27(2), 72-80.

6. Temiz Ö. (2020) Biopesticide emamectin benzoate in the liver of male mice: evaluation of oxidative toxicity with stress protein, DNA oxidation, and apoptosis biomarkers, ESPR, 27(18), 23199-23205.

7. Lu J. et al. (2022) Induction of developmental toxicity and cardiotoxicity in zebrafish embryos by Emamectin benzoate through oxidative stress, Sci. Total Environ., 825, 154040.

8. Wei Z. et al. (2023) The potential immunotoxicity of emamectin benzoate on the human THP-1 macrophages, Environ. Toxicol., 38(3), 500-510.

9. Gabr G., Abdulaziz S.S., Al-Kahtani A.A., Ali B.E. (2015) Teratogenic effects in rat fetuses subjected to the concurrent in utero exposure to emamectin benzoate insecticide, PJBS, 18(7), 333-340,.

10. Abou-Zeid S.M., AbuBakr H.O., Mohamed M.A., El-Bahrawy A. (2018) Ameliorative effect of pumpkin seed oil against emamectin induced toxicity in mice, Biomed. Pharmacother., 98, 242-251.

11. Zhang Y. et al. (2020) Effect of a beta-cypermethrin and emamectin benzoate pesticide mixture on reproductive toxicity in male mice in a greenhouse environment, Toxicol. Mech. Methods, 30(2), 100-106.

12. Bowker J.D., Carty D., Bowman M.P. (2013) The safety of SLICE (0.2% emamectin benzoate) administered in feed to fingerling rainbow trout, N. Am. J. Aquac., 75(4), 455-462.

13. Julinta R. et al. (2020) Effects of oral-dosing of an antiparasitic drug emamectin benzoate on the growth and serum biomarkers of *Oreochromis niloticus* (L.) juveniles, J. Environ. Biol., 41(5), 973-979.

14. Choudhary P. et al. (2022) Effect of graded levels of dietary emamectin benzoate on immunity, enzyme activity, and withdrawal period in *Labeo rohita* Juveniles (Hamilton, 1822), Aquac. Nutr., 2022.

15. Mobeen A., Ishrat I., Awan F.R., Mansoor S., Khan Q.M. (2022) *In vivo* toxicity assessment of biopesticide emamectin benzoate and its commercially used formulations in Pakistan, J Pharm. Pharmacol., 10, 1-14.

16. Hong X., Zhao X., Tian X., Li J., Zha J. (2018) Changes of hematological and biochemical parameters revealed genotoxicity and immunotoxicity of neonicotinoids on Chinese rare minnows (*Gobiocypris rarus*), Environ Pollut., 233, 862-871.

17. Kumar V. et al. (2022) Assessment of the effect of sub-lethal acute toxicity of Emamectin benzoate in *Labeo rohita* using multiple biomarker approach, Toxicol. Rep., 9, 102-110.

18. Fetoui H., Makni M., Garoui E.M., Zeghal N. (2010) Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid, Exp. Toxicol. Pathol., 62(6), 593-599.

19. Akmal H., Ahmad S., Akram I. (2023) Assessment of water quality and targeted accumulation of some heavy metals in different organs of fresh water fish ompok bimaculatus in riverine system of Punjab, Pakistan: assessment of water quality, PBMJ, 24-29.

20. Mahmood A., Ahmad S., Akmal H., Shahzad K. (2023) Evaluation of hemotoxic, hepatotoxic and nephrotoxic potential of profenofos-based insecticide in freshwater *Labeo rohita* fish at low concentrations: evaluation of profenofos-based insecticide's potential, PBMJ, 32-40.

21. Akmal H. et al. (2023) Morphometry and food preference in relation to sex and hematological values of Eurasian collared dove (*Streptotella decaocto*), JAVAR, 10(2), 244.

22. Ahmad S., Akmal H., Jafar K. (2023) A comparative study of morphometric profiles and hematological analysis and food preference between male and female spotted redshank (*Tringa erythropus*) sampled from different wetlands of Punjab, Pakistan, RADS J. Biol. Res. Appl. Sci, 14(1).

23. Alghamdi S.A. (2020) Effect of *Nigella sativa* and *Foeniculum vulgare* seeds extracts on male mice exposed to carbendazim, Saudi J. Biol. Sci., 27(10), 2521-2530.

24. Suzana D., Suyatna F.D., Andrajati R., Sari S.P., Mun'im A. (2017) Effect of *Moringa oleifera* leaves extract against hematology and blood biochemical value of patients with iron deficiency anemia, JYP, 9(1), S79.

25. Ncha O.S., Michael P.B., Nnabuchi U.O., Alex E. (2015) Effect of diets with moringa leaf meal on growth, carcass composition and haematology of *Clarias gariepinus*, Int. J. Fish. Aquat., 3(2), 397-401.

26. Bbole I., Mumba C., Mupenda N., Kefi A.S. (2016) Analysis of growth performance and haematological parameters of Oreochromis niloticus fed on a varying diet of *Moringa oleifera* Lam. leaf meal as an additive protein source, Int. J. Fish. Aquac., 8(11), 105-111.

27. Kavitha C., Ramesh M., Kumaran S.S., Lakshmi S.A. (2012) Toxicity of *Moringa oleifera* seed extract on some hematological and biochemical profiles in a freshwater fish, *Cyprinus carpio*, 64(7-8), 681-687.

28. Dzoyem J.P., Kuete V., Eloff J.N. (2014) Biochemical parameters in toxicological studies in Africa: significance, principle of methods, data interpretation, and use in plant screenings. In: Toxicological survey of African medicinal plants: Elsevier, pp. 659-715.

29. Lee D.-C., Choi Y.J., Kim J.-H. (2022) Toxic effects of waterborne cadmium exposure on hematological parameters, oxidative stress, neurotoxicity, and heat shock protein 70 in juvenile olive flounder, *Paralichthys olivaceus*, Fish Shellfish Immunol., 122, 476-483.

30. Tang Y. et al. (2022) Sensitive determination of metalloprotein in salt-rich matrices by size exclusion chromatography coupled with inductively coupled plasma-mass spectrometry, J. Chromatogr. A, 1677, 463303.

31. Azoz A., Ibrahim K.A., Abdel Kader I., Tawfik A. (2020) Tracking of apoptosis induced by emamectin benzoate in fetuses of hypothyroid rats, Int J Pharm Sci Rev Res., 13, 81-89.

32. Naik R.A., Rawat D., Ahi J.D., Koiri R.K. (2021) Ameliorative effect of piracetam on emamectin benzoate induced perturbations in the activity of lactate dehydrogenase in murine system, Advances in Redox Research, 3, 100019.

33. Afsar T., Razak S. (2017) Modulatory influence of *Acacia hydaspica* R. Parker ethyl acetate extract against cisplatin inveigled hepatic injury and dyslipidemia in rats, BMC Complement Altern Med., 17(1), 1-13.

34. El-Kassas S. et al. (2020) Growth performance, serum lipid profile, intestinal morphometry, and growth and lipid indicator gene expression analysis of mono-sex Nile tilapia fed *Moringa oleifera* leaf powder, Fish Shellfish Immunol., 18, 100422.

35. El-bakry K., Toson E.-s., Serag M., Aboser M. (2016) Hepatoprotective effect of Moringa oleifera leaves extract against carbon tetrachloride-induced liver damage in rats, World J Pharm Sci., 5(5), 76-89.

36. Raibeemol K., Chitra K. Induction of immunological, hormonal and histological alterations after sublethal exposure of chlorpyrifos in the freshwater fish, *Pseudetroplus maculatus* (Bloch, 1795)," *Fish & shellfish immunology,* vol. 102, pp. 1-12, 2020.

37. Akmal H., Ahmad S., Abbasi M.H., Jabeen F., Shahzad K. (2024) A study on assessing the toxic effects of ethyl paraben on rohu (Labeo rohita) using different biomarkers; hemato-biochemical assays, histology, oxidant and antioxidant activity and genotoxicity, PloS one, 19(5), e0302691.

38. Ashour E.A. et al. (2020) Effect of dietary supplementation with *Moringa oleifera* leaves and/or seeds powder on production, egg characteristics, hatchability and blood chemistry of laying Japanese quails, Sustainability, 12(6), 2463.

Information about authors:
Iqra Jamil – Mphil, Department of Zoology, University of Okara (Okara, Pakistan, e-mail: hafizaiqrajamil@gmail.com)
Shabbir Ahmad – Mphil, Department of Zoology, University of Okara (Okara, Pakistan, e-mail: shabbir35303@gmail.com)
Hasnain Akmal – Mphil, Department of Zoology, University of Okara (Okara, Pakistan, e-mail: hasnain20156@gmail.com)
Iqra Akram– Mphil, Department of Zoology, University of Okara (Okara, Pakistan, e-mail: iqraakramhayat@gmail.com)
Muqadas Shahzadi – Mphil, Department of Zoology, University of Okara (Okara, Pakistan, e-mail: muqadas54111@gmail.com)
Sajid Ali – Mphil, Department of Zoology, University of Okara (Okara, Pakistan, e-mail: abidalisahibzada@gmail.com)
Sheraz Khan – Mphil, Department of Zoology, University of Okara (Okara, Pakistan, e-mail: sherazk0073@gmail.com)
Khurram Shahzad (corresponding author) – PhD, Assistant Professor, Department of Zoology, University of Okara (Okara, Pakistan, e-mail: dr.khurram@uo.edu.pk)