Archives of Ecotoxicology, Vol. 4, No. 4, pp. 94-104, 2022

SCICELL

Archives of Ecotoxicology

Journal homepage: https://office.scicell.org/index.php/AE

OPEN ACCESS

Histopathological Alterations in Nile Tilapia *Oreochromis Niloticus* (L.) as Influenced by Dietary Antiparasitic Drug Emamectin Benzoate at the Recommended and Higher Doses for an Extended Period

Thangapalam Jawahar Abraham[a]*, Roy Beryl Julinta[a], and Prasanna Kumar Patil[b]

[a] *Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata 700094, India*
[b] *Aquatic Animal Health and Environment Division, ICAR-Central Institute of Brackishwater Aquaculture, Chennai 600028, India*

Article info
Received 27 November 2022
Revised 23 December 2022
Accepted 24 December 2022
Published online 31 December 2022

Regular article

Keywords:
Aquaculture
Fish health
Parasitic diseases
Antiparasitic medication
Biosafety
Histopathology

Abstract

The effects of the dietary antiparasitic drug emamectin benzoate (EB) on the biosafety and kidney, liver and intestine tissue histopathology of *Oreochromis niloticus* were assessed. Extended EB medication at the recommended dose (50 µg/kg biomass/day) for 21 days reduced survival, feed intake and biomass. A dose-dependent hepatotoxic, nephrotoxic and intestinal toxic effects of EB were noticed. Tubular degeneration, glomerulopathy, inflammation and necrotized renal interstitium in the kidney and glycogen-type vacuolation and cytoplasmic degeneration in the liver were observed. The intestine exhibited loss of absorptive vacuoles, mucinous degeneration, necrotized intestinal villi and absorptive region and inflammation. Lamina propria swelling was noticed in the higher-dosed groups. With the termination of medication, the pathological changes were reduced significantly, indicating the ability of fish to mount adaptive responses to recoup. The extended EB administration for 21 days is unlikely to produce adverse and irrevocable effects on fish at the recommended dose.

1. Introduction

The rapid expansion of aquaculture has catalyzed the growth of the tilapia industry in most Southeast Asian countries. Tilapias are second only to carps as the most popular farmed freshwater fish in the world **(FAO, 2020)**. Owing to technological breakthroughs, the potential of tilapias as cultivable species is increasingly recognized due to their inherent advantages such as tasty flesh, fewer bones, quick growth, ease of reproduction, ready acceptance of artificial feed, and effectiveness in controlling insects and weeds **(Prabu *et al.,* 2019)**. Diseases are always a threat and can result in significant stock losses, affecting fish supply and farmers' livelihoods. Tilapias are expected to be resistant to several diseases that affect other farmed fish, but many diseases of bacterial and viral origin have been reported **(Zamri-Saad *et al.,* 2014; Behera *et al.,* 2018; Jansen *et al.,* 2019)**. Challenges to aquaculture include animal welfare, environmental perturbation, and ecological impacts besides diseases **(González-Gaya *et al.,* 2022)**. In aquaculture, disease control is achieved through a combination of preventive measures, good management practices, and the use of approved, appropriate and effective drugs and vaccines. The control of diseases caused by bacteria, fungi, parasites, and viruses demands the judicious use of aquadrugs **(Patil *et al.,* 2022)**. In fish farming, medicated feeds allow continuous fish growth, if it is administered according to label directions at the early onset of disease while fish are still appetent **(Kumar and Gaunt, 2020)**.

The most extensively used approach to managing parasite infections in cultured aquatic species is anti-parasitic medication. Despite significant investment in the development of new anti-parasitic medications, the number of effective medicines now available for users worldwide is restricted **(Sanders and Swan, 2014)**. Emamectin benzoate (EB) is a close relative to ivermectin of the avermectin family and is produced by *Streptomyces avermitilis* **(Moschou *et al.,* 2019)**. It is formulated as SLICE® for the control of sea lice **(Sanders and Swan, 2014)**. The avermectins' main mechanism of action is in the nervous system by interrupting the transmission of impulses. They interact with many ligand-gated chloride channels to increase the permeability of the cell membrane and finally cause nervous system malfunction **(USDA, 2010; Singha *et al.,* 2022)**. It has become the preferred treatment of choice as it is efficacious against all life stages and has a long-lasting effect. Unlike labour-intensive and stressful bath treatments, EB as an in-feed therapeutant is easily, safely and effectively administered **(Lees *et al.,* 2008)**. The commercial product of EB, SLICE®, has been extensively evaluated for its environmental safety, efficacy, and tolerance in Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, and brown trout *Salmo trutta* in the marine environment **(Armstrong *et al.,* 2000; Roy *et al.,* 2000)**.

Crustacean ectoparasites are identified as an important health problem in salmon aquaculture (Nilsen et al. 2017; Powell et al. 2018). Alike, severe economic losses in carp aquaculture due to

*Corresponding author: abrahamtj1@gmail.com
https://doi.org/10.36547/ae.2022.4.4.94-104

infestation by fish lice *Argulus* spp., have been documented **(Sahoo *et al.,* 2013)**. In Indian aquaculture, antiparasitic agents like deltamethrin, amitraz, quinolphos, albendazole, ivermectin, and cypermethrin are applied to control *Argulus* spp., *Caligus* spp., *Lernaea* spp., and *Lernanthropsis* spp., infestations **(Patil *et al.,* 2022)**. Though Indian agricultural producers use EB as a pesticide to manage crop pests **(Anon, 2021)**, its use in aquaculture is yet to pick up. Documentations are available on the application of EB and its effectiveness in controlling parasites **(St-Hilaire *et al.,* 2021; Anandaraja *et al.,* 2022)** and on the safety of tropical fish **(Julinta *et al.,* 2020a, 2020b; Anandaraja *et al.,* 2021; 2022; Singha *et al.,* 2022; Das *et al.,* 2022)**. No systematic reports are, however, available on its effect on the histopathology of commercially cultured tropical fish. This study was, therefore, taken up to assess the effects of an antiparasitic agent emamectin benzoate on the biosafety and kidney, liver, and intestine tissue histopathology of *Oreochromis niloticus,* when administered orally.

2. Material and methods

2.1 Experimental fish

Active Nile tilapia *Oreochromis niloticus* juveniles obtained from a farm (Lat. 22°27′50.2158″ N; Long. 88°23′7.4004″ E), West Bengal, India were transported to the laboratory in oxygen-filled polythene bags. The fish were given an immersion treatment in 5 ppm potassium permanganate solution for 5 min and transferred to five circular fibreglass reinforced plastic tanks (FRP) at 90 numbers/tank holding 400 L of aerated bore-well water. A floating pellet diet (CP Private Limited, India) was offered to fish to satiation twice daily. The fish were accustomed to the laboratory conditions for 3 weeks with continuous aeration and replacement of 50% water twice weekly to avoid waste build-up. The water quality parameters were maintained at optimal levels (temperature: 22.00–30.00°C, pH: 7.80–8.60, dissolved oxygen: 4.90–5.20 mg/L, ammonia: 0.002–0.008 mg/L, nitrite: 0.14–0.53 mg/L and nitrate: 0.13–0.55 mg/L) throughout the experimental period.

2.2 Emamectin Benzoate Diets Preparation

The emamectin benzoate (EB), as Slice®, is administered at 50 µg/kg biomass/day for 7 consecutive days as an in-feed therapeutant against fish ectoparasites **(MSD Animal Health, 2012)**. In this study, the biosafety of EB (Sigma-Aldrich, India) was assessed by feeding *O. niloticus* with top-coated diets at 0-10 times the recommended dose (1×: 50 µg/kg biomass/day) and 3 times the dosage, i.e., 3 × 7 days. The EB diets for feeding the fish at 2% of the body weight (BW) at a dose of 50 µg (1×), 125 µg (2.5×), 250 µg (5×), and 500 µg/kg biomass/day (10×) were prepared as described earlier **(Julinta *et al.,* 2020a,b; Singha *et al.,* 2022)**. In brief, the required volume of the EB stock solution was added to 5.0 mL refined sunflower oil and then admixed with a 1 kg dry basal pellet diet in order of increasing concentration. The control diet without EB was prepared following the above procedure, air-dried overnight, and stored in separate airtight plastic containers.

2.3 Experimental Design and Dose Administration

Fifteen tanks (L58 × H45 × W45 cm) with 20 fish (12.85 ± 1.00 g) each were serially numbered and allocated into 5 groups, namely, group 1: 0× control diet, group 2: 1× EB-diet (50 µg), group 3: 2.5× EB-diet (125 µg), group 4: 5× EB-diet (250 µg) and group 5: 10× EB-diet (500 µg), in triplicates. Before the transfer, a group of 10 fish from each group were individually weighed. Group 1 offered the control diet. The fish of groups 2 - 5 were fed the control diet during the pre-dosing period (days 1 - 7). During the EB-dosing (ED) period (days 8 - 28), groups 2 - 5 were fed with the respective EB diets at 2% BW thrice daily to achieve an EB dose of 50 µg, 125 µg, 250 µg, and 500 µg/kg biomass/day. During the post-EB-dosing (PED) period from day 29 to 42 (14 days), all fish groups were fed the control diet. For each tank based on biomass, the quantity of diet offered was planned weekly. The floating pelleted diets lasting in the tank 60 min after each feeding were siphoned into a pre-weighed vessel, dried overnight, and weighed. The feeding behaviour based on the feed consumed by the fish was noted daily and given numerical scores on a five-point ordinal scale, i.e., 0 = No feed consumed; 1 = Approximately 25% of feed consumed; 2 = Approximately 50% of feed consumed; 3 = Approximately 75% of feed consumed and 4 = Approximately 100% of feed consumed **(Bowker *et al.,* 2013)**. The mortalities, fish behavioural changes like lethargy, aggressive and subdued feeding, position in the water column, equilibrium loss, uncharacteristic pigmentation or discolouration, gasping for air, flashing, hyperactivity, and other gross and dermal lesions were observed daily.

2.4 Histopathology

On day 0 and day 21 ED and day 14 PED, two fish from each tank were collected and euthanized using clove oil at 60 µL/L water **(AVMA, 2020)**. The euthanized fish were carefully dissected, removed a portion of the mid kidney, liver, and mid intestine was gently rinsed in saline and fixed in Bouin's fixative for 24 h. Further processing of tissues, such as dehydration in ascending grades of ethyl alcohol (50 - 100%), clearing in xylene (2 changes), embedding in molten paraffin, sectioning, staining and histopathological analyses were as described in **Roberts (2012)**. Digital microphotographs were taken in an Olympus microscope BX51 using a 16 MP SCO-LUX camera attached to the microscope and computer. The histopathological changes in different organs of EB-dosed *O. niloticus* were evaluated based on the severity in comparison with the control. The major changes were identified and given a qualitative assessment score using an ordinal scale, according to the percentage alteration of the tissue due to damages from its normal architecture, viz., 0 = No change; 1 = Normal with <5% of tissues affected; 2 = Mild with 5 – 15% of tissues affected; 3 = Moderate with 15 – 25% of tissues affected; 4 = Marked with 25 – 50% of tissues affected and 5 = Severe with >50% of tissues affected (Bowker et al. 2013). The investigational protocols fulfilled the ethical guidelines including observance of the legal requirements of India **(CPCSEA, 2021)**.

2.5 Statistical Analyses

The completely randomized experimental design results are presented as mean ± standard deviation. The data on survival and biomass were analysed by one-way ANOVA and Tukey HSD posthoc for the comparison of means. The feeding behaviour numerical scores and qualitative scores of histopathological changes were assessed by Kruskal–Wallis test with pair-wise comparisons. All tests were analysed using the Statistical Package for Social Sciences (IBM-SPSS) Version: 22.0, considering a probability level of $P < 0.05$.

3. Results

3.1 Feeding behaviour, survival, biomass, fish behaviour and abnormalities

The feeding activities of the control were normal. The full portions of pre-dosing feed were consumed and the feeding

behaviour was normal. During the ED period, a significant reduction in feed intake was noticed in all the groups ($P < 0.05$). With the termination of dosing, an enhanced feed intake was noted in all groups. Also, significant differences in feed intake existed among the dosing periods in all groups ($P < 0.05$). Mortalities were recorded, though insignificant, in all ED groups during the dosing regimen ($P > 0.05$). No death was noticed during the PED period, except for the 10× group (Table 1). The ED caused a significant dose-dependent reduction in weight increment ($P < 0.05$) in the dosing groups compared to the control, except for the 1× group (Table 1) in 42 days. The weight increment among the higher dosing groups differed insignificantly ($P>0.05$). The lower-dosed group of fish exhibited no flashing and hyperactivity and were distributed throughout the water column. The fish of the 5× and 10× groups showed loss of equilibrium, darkened body colour, and mucus-associated gills with reddening during the dosing period. Necropsy was performed on freshly dead fish. The fish of the 2.5× - 10× groups had swollen and discoloured kidneys, hepatomegaly, splenomegaly and swollen intestine. No external abnormalities were observed on day 14 of PED and all fish became almost normal.

Table 1 Biosafety of emamectin benzoate (EB)-dosed *Oreochromis niloticus* juveniles at 0-10 times the recommended dose of 50 µg/kg biomass/day (1×) for 21 consecutive days

Parameters	EB diets				
	0 µg (0×)	50 µg (1×)	125 µg (2.5×)	250 µg (5×)	500 µg (10×)
Feed intake score*					
Pre-dosing period, 0 - 7 days	4.00±0.00	4.00±0.00[1]	4.00±0.00[1]	4.00±0.00[1]	4.00±0.00[1]
Dosing period, 8 - 28 days	4.00±0.00[a]	3.00±0.63[b2]	2.90±0.54[b2]	2.62±0.49[bc2]	2.19±0.40[c2]
Post-dosing period, 29 - 42 days	4.00±0.00	3.92±0.26[1]	3.92±0.26[1]	3.86±0.36[1]	3.72±0.47[1]
Survival (%)**	100.00±0.00	98.33±2.87	98.33±2.87	98.33±2.87	95.00±5.00
Biomass: Weight increment (g)#	70.00±5.00[a]	66.33±1.53[a]	53.33±4.51[b]	55.00±4.00[b]	49.33±2.52[b]

a-c: Values sharing uncommon alphabets within a row differed significantly ($P < 0.05$). 1-2: Values sharing uncommon numerals within the column differed significantly ($P < 0.05$). *Five-point ordinal scale: 0 = No feed consumed; 1 = Approximately 25% of feed consumed; 2 = Approximately 50% of feed consumed; 3 = Approximately 75% of feed consumed and 4 = Approximately 100% of feed consumed. **: On day 42. #: Final weight – Initial weight.

3.2 Histopathology

The control group exhibited normal kidney, liver, and intestinal tissue architecture (Figures 1a-c). A significant dose-dependent increase in the histopathological changes ($P < 0.05$) like degeneration of the renal tubules and tubular epithelium, vacuolation and inflammation of the renal tubules, glomerulopathy indicating shrunken glomerulus and/or fragmented glomerulus with dilated Bowman's space and necrotized renal interstitium was observed in the dosing groups on day 21 of ED. In addition, the thickening of the lumen lining was noticed (Figures 2a,c,e,g). Necrosis and necrotized renal interstitium were more prominent in the 10× group (Figure 2g). The extent of kidney tissue damage was, however, reduced significantly ($P<0.05$) on day 14 of PED in a dose-dependent manner (Figures 2b,d,f,h; Table 2). Severe glycogen-type vacuolation was noticed on day 21 ED in all the dosing groups, with significantly high intensities ($P < 0.05$) in the 5× and 10× groups (Figures 3a,c,e,g). The other common changes noted in the liver tissues were dose-dependent cytoplasmic degeneration. Though the damages were relatively low compared to the glycogen-type vacuolation, the extent of damage was significant among the dosing groups ($P < 0.05$). A significant mild but dose-dependent increase ($P<0.05$) in cytoplasmic degeneration was observed in 2.5× - 10× groups (Figures 3d,f,h). Besides, the liver tissues of *O. niloticus* exhibited dilation of blood vessels in the higher-dosed groups. In most of the groups, with the suspension of dosing, the extent of liver tissue damage was, however, reduced significantly ($P < 0.05$) in a dose-dependent manner (Figures 3b,d,f,h; Table 2). The absorptive vacuole loss, mucinous degeneration, necrotized intestinal villi, inflammation, and absorptive region necrosis were the common intestinal histopathological changes in the 1× group (Figure 4a). Besides these changes, mild mucinous degeneration and degeneration of the epithelial layer were noted in the 2.5× - 10× groups on day 21 ED (Figures 4c,e,g), with a dose-dependent increase ($P < 0.05$). The intestine of the 5× and 10× groups had lamina propria swelling as the distinctive change (Figures 4e-h). A dose-dependent significant reduction ($P < 0.05$) in the intensity of most of the intestinal tissue damage was noticed on day 14 of PED in all the groups (Figures 4b,d,f,h; Table 2).

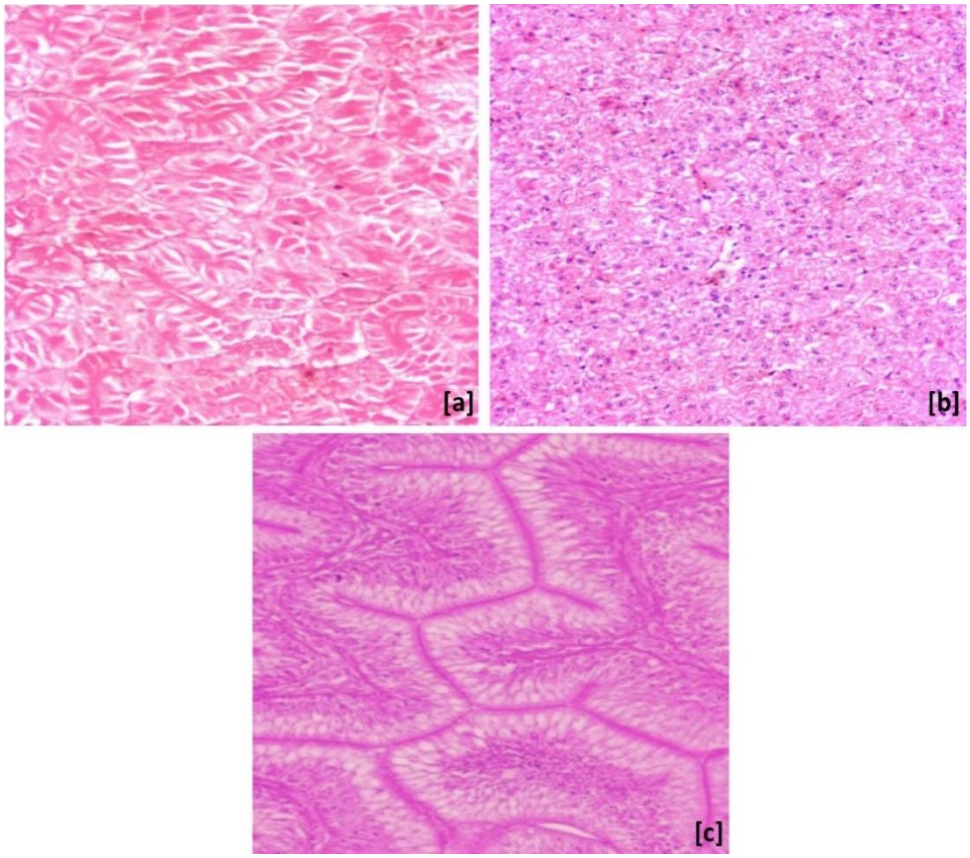

Figure 1 Histoarchitecture of the control diet fed *Oreochromis niloticus* juveniles showing normal [a] kidney; [b] liver and [c] intestine, ×200 H&E staining.

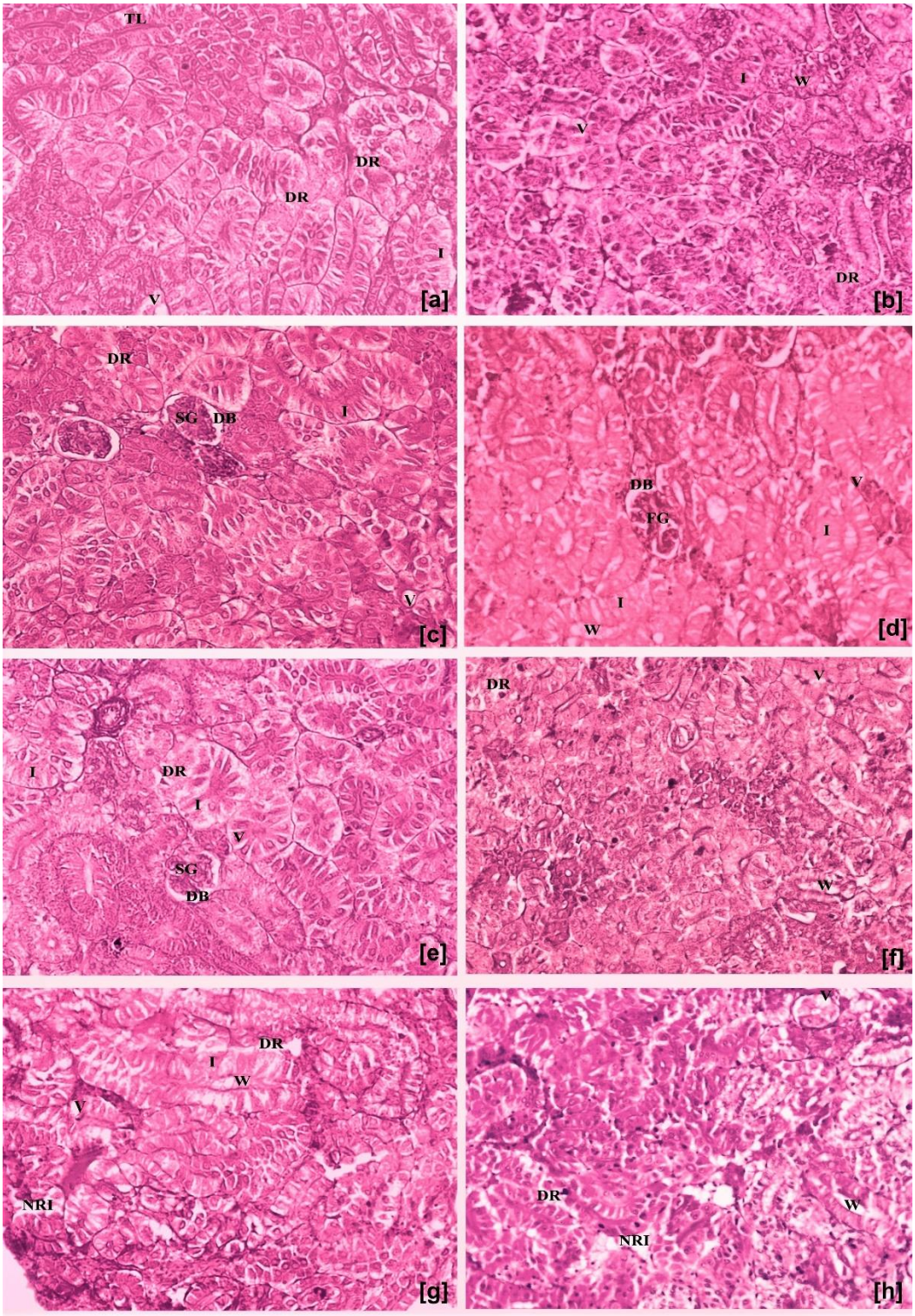

Figure 2 Histopathological changes in the kidney tissues of emamectin benzoate (EB)-dosed *Oreochromis niloticus* juveniles for 21 consecutive days [a, b] at 50 µg/kg biomass/day (1× group) [a] on day 21 EB-dosing (ED), ×200; [b] day 14 post-EB-dosing (PED), ×200; [c, d] at 125 µg/kg biomass/day (2.5× group) [c] on day 21 ED, ×200; [d] day 14 PED, ×200; [e, f] at 250 µg/kg biomass/day (5× group) [e] on day 21 ED, ×200; [f] day 14 PED, ×200; and [g, h] at 500 µg/kg biomass/day (10× group) [g] on day 21 ED, ×200; [h] day 14 PED, ×200 showing degeneration of renal tubular epithelium (DR), thickening of lumen lining (TL), and renal tubules with vacuolation (V), inflammation (I), widened lumen (W), shrunken glomerulus (SG) with dilated Bowman's space (DB), fragmented glomerulus (FG), and necrotized renal interstitium (NRI), H&E staining.

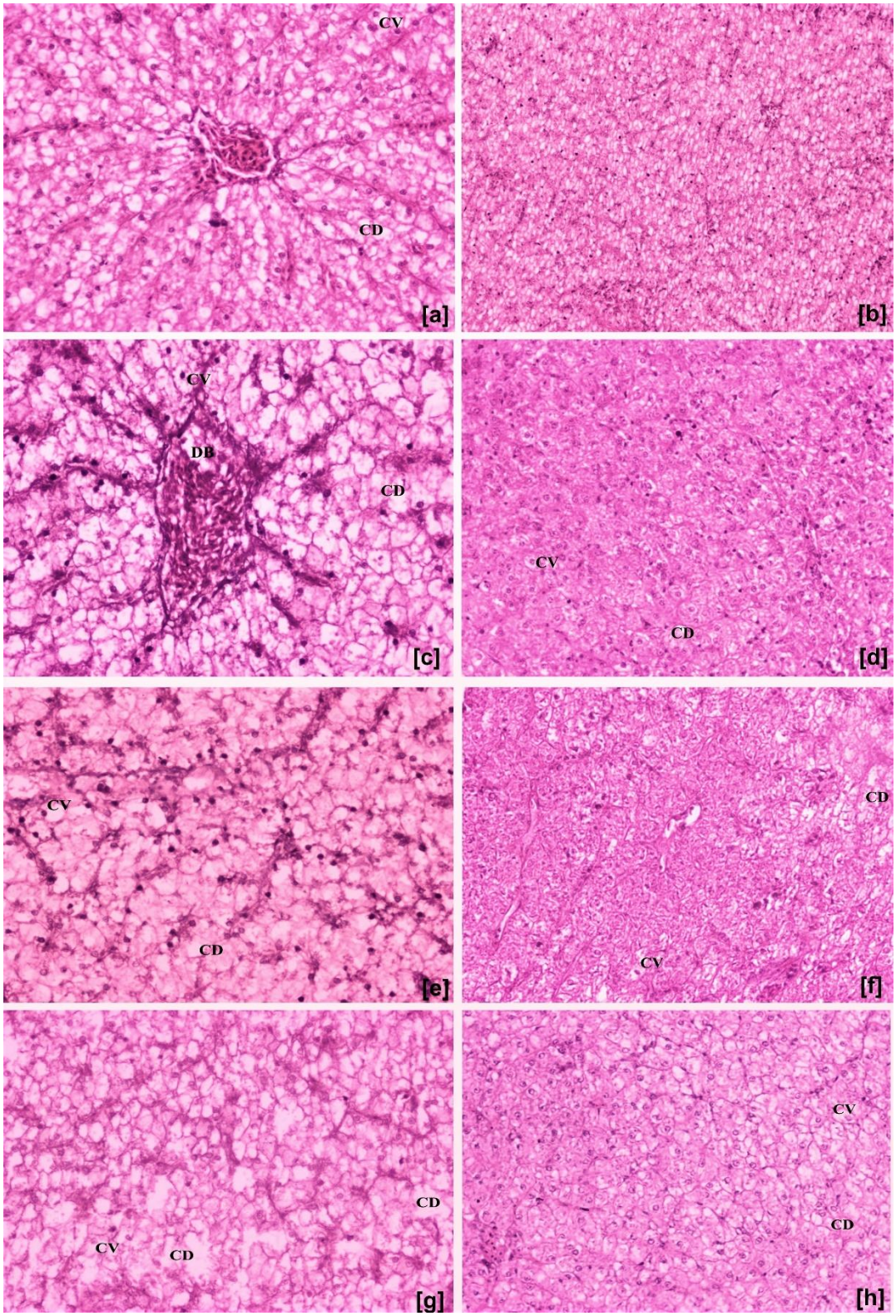

Figure 3 Histopathological changes in the liver tissues of emamectin benzoate (EB)-dosed *Oreochromis niloticus* juveniles for 21 consecutive days [a, b] at 50 µg/kg biomass/day (1× group) [a] on day 21 EB-dosing (ED), ×200; [b] day 14 post-EB-dosing (PED), ×100; [c, d] at 125 µg/kg biomass/day (2.5× group) [c] on day 21 ED, ×200; [d] day 14 PED, ×200; [e, f] at 250 µg/kg biomass/day (5× group) [e] on day 21 ED, ×200; [f] day 14 PED, ×200; and [g, h] at 500 µg/kg biomass/day (10× group) [g] on day 21 ED, ×200; [h] day 14 PED, ×200 showing glycogen-type vacuolation, cytoplasmic vacuolation (CV) and cytoplasm degeneration (CD), and dilated blood vessel (DB), H&E staining.

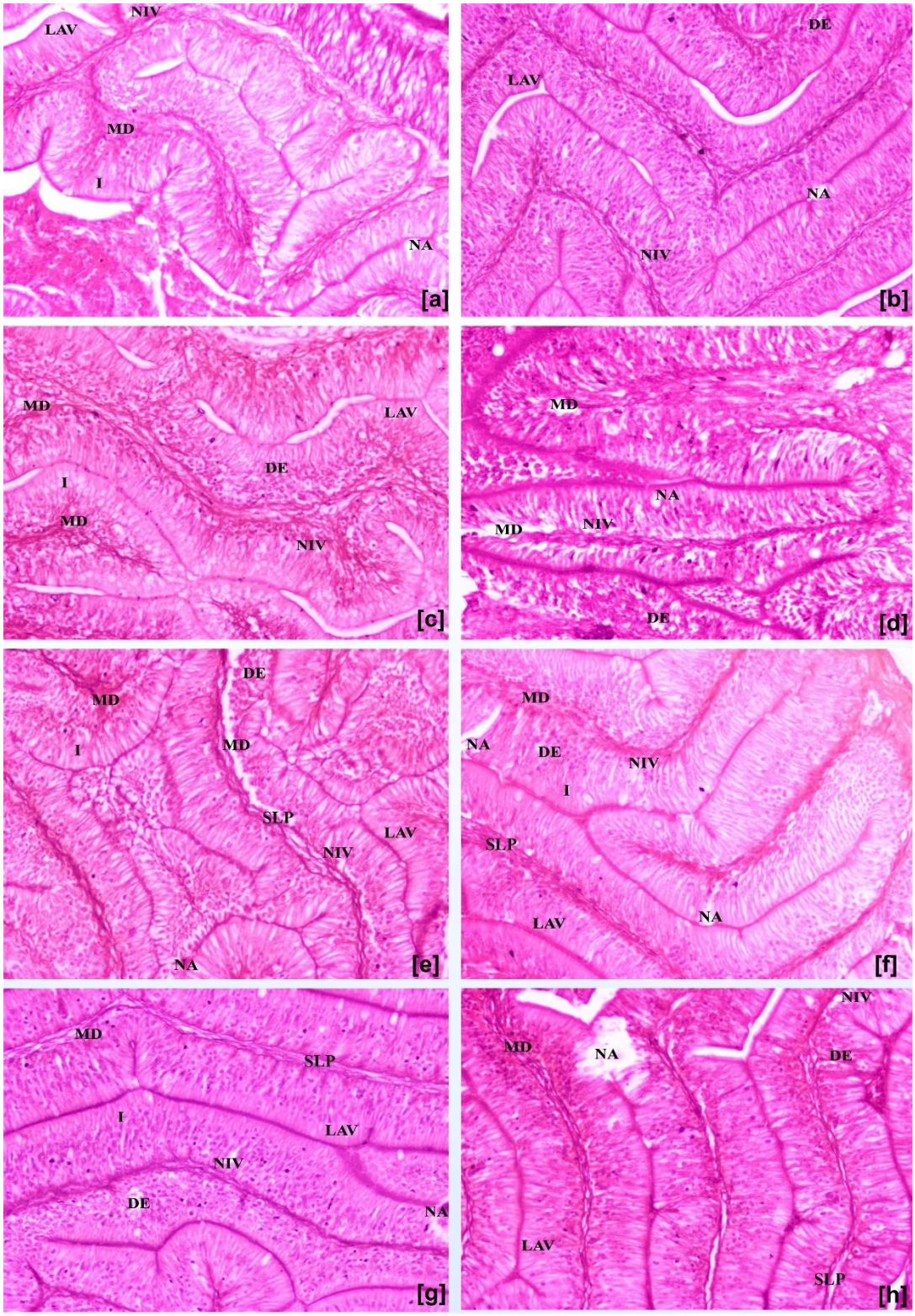

Figure 4 Histopathological changes in the intestinal tissues of emamectin benzoate (EB)-dosed *Oreochromis niloticus* juveniles for 21 consecutive days [a, b] at 50 µg/kg biomass/day (1× group) [a] on day 21 EB-dosing (ED), ×200; [b] day 14 post-EB-dosing (PED), ×200; [c, d] at 125 µg/kg biomass/day (2.5× group) [c] on day 21 ED, ×200; [d] day 14 PED, ×200; [e, f] at 250 µg/kg biomass/day (5× group) [e] on day 21 ED, ×200; [f] day 14 PED, ×200; and [g, h] at 500 µg/kg biomass/day (10× group) [g] on day 21 ED, ×200; [h] day 14 PED, ×200 showing mucinous degeneration (MD), necrosis in the intestinal villi (NIV), necrotised absorptive region (NA), loss of absorptive vacuoles (LAV), inflammation (I), degeneration of the epithelial layer (DE) and swollen lamina propria (SLP) H&E staining

Table 2 Qualitative assessment of the major histopathological changes in emamectin benzoate (EB)-dosed *Oreochromis niloticus* juveniles at 0-10 times the recommended dose of 50 µg/kg biomass/day (1×) for 21 consecutive days in comparison with the control

Histopathological changes	Qualitative assessment on a five-point ordinal scale*							
	50 µg (1×)		125 µg (2.5×)		250 µg (5×)		500 µg (10×)	
	ED	PED	ED	PED	ED	PED	ED	PED
Kidney								
Degeneration of renal tubular epithelium + tubules	2.06±0.11^{1a}	1.91±0.16^{2b}	2.14±0.11^{1a}	1.92±0.21^{2b}	2.33±0.10^{1c}	2.30±0.09^{1c}	3.21±0.10^{1d}	2.79±0.16^{2e}
Renal tubules inflammation	1.38±0.08^{1a}	0.60±0.11^{2b}	1.52±0.03^{1c}	0.62±0.15^{2b}	1.60±0.10^{1c}	0.63±0.12^{2b}	1.61±0.12^{1c}	0.65±0.05^{2b}
Vacuolation	1.14±0.09^{1a}	0.80±0.01^{2b}	1.17±0.10^{1a}	0.81±0.05^{2b}	1.36±0.07^{1c}	0.81±0.18^{2b}	1.46±0.06^{1d}	0.83±0.15^{2b}
Necrotized renal interstitium	0.23±0.08^{1a}	0.23±0.08^{1a}	0.23±0.08^{1a}	0.23±0.08^{1a}	0.30±0.11^{1b}	0.30±0.11^{1b}	1.12±0.17^{1c}	0.89±0.15^{2d}
Glomerulopathy#	0.42±0.04^{1a}	0.35±0.08^{1a}	1.07±0.12^{1b}	0.92±0.08^{2c}	1.13±0.08^{1bd}	0.98±0.04^{2ce}	1.17±0.12^{1d}	1.02±0.18^{2be}
Liver								
Glycogen-type vacuolation	4.52±0.04^{1a}	3.17±0.08^{2b}	4.58±0.04^{1a}	3.18±0.04^{2b}	4.60±0.09^{1ac}	3.23±0.05^{2b}	4.68±0.04^{1c}	3.40±0.13^{2d}
Cytoplasmic vacuolation	0.37±0.08^{1a}	0.30±0.11^{1a}	0.37±0.08^{1a}	0.30±0.11^{1a}	0.47±0.10^{1b}	0.37±0.08^{2a}	0.63±0.08^{1c}	0.47±0.10^{2b}
Cytoplasmic degeneration	0.90±0.11^{1a}	0.37±0.08^{1b}	1.11±0.06^{1c}	0.38±0.10^{2b}	1.34±0.20^{1d}	0.99±0.05^{2a}	1.47±0.04^{1e}	1.19±0.08^{2c}
Intestine								
Loss of absorptive vacuoles	2.70±0.11^{1a}	2.26±0.17^{2b}	2.82±0.11^{1c}	2.64±0.05^{2a}	2.83±0.05^{1c}	2.65±0.10^{2a}	2.84±0.13^{1c}	2.67±0.08^{2a}
Inflammation	1.05±0.08^{1a}	0.92±0.13^{2b}	1.24±0.06^{1ce}	1.03±0.05^{2a}	1.32±0.09^{1ce}	1.21±0.07^{2c}	1.43±0.11^{1d}	1.26±0.09^{2ce}
Mucinous degeneration	1.10±0.08^{1a}	0.73±0.10^{2b}	1.28±0.08^{1c}	1.20±0.03^{1c}	1.38±0.07^{1de}	1.23±0.07^{1c}	1.43±0.04^{1d}	1.29±0.07^{2ce}
Degeneration of the epithelial layer	0.28±0.10^{1a}	0.23±0.08^{1a}	0.42±0.04^{1b}	0.32±0.13^{2d}	0.55±0.12^{1c}	0.33±0.10^{2bd}	0.62±0.04^{1c}	0.40±0.06^{2b}
Necrosis of absorptive region	0.96±0.11^{1a}	0.67±0.16^{2b}	0.98±0.06^{1a}	0.83±0.65^{2c}	1.08±0.08^{1de}	1.02±0.04^{1d}	1.15±0.05^{1e}	1.08±0.04^{1d}
Necrosis of intestinal villi	0.90±0.11^{1a}	0.67±0.16^{2b}	1.25±0.06^{1cd}	1.17±0.04^{1c}	1.31±0.07^{1d}	1.18±0.07^{2c}	1.39±0.05^{1d}	1.19±0.08^{2c}

*: Qualitative assessment ordinal scale: 0 = No change; 1 = Normal with <5% of tissues affected; 2 = Mild with 5 – 15% of tissues affected; 3 = Moderate with 15 – 25% of tissues affected; 4 = Marked with 25 – 50% of tissues affected and 5 = Severe with >50% of tissues affected. The qualitative assessment was based on six observations (mean ± standard deviation) for each organ of the respective group. No changes were noted in the control group. 1-2: Values sharing uncommon numerical superscripts within a row for a particular histopathological change and a particular treatment (dose) differed significantly ($P < 0.05$). a-e: Values sharing uncommon alphabetical superscripts for a particular row among the treatments (doses) differed significantly ($P < 0.05$). ED: Day 21 EB-dosing; PED: Day 14 post-EB-dosing. #Glomerulopathy includes shrunken glomerulus and/or fragmented glomerulus with dilated Bowman's space.

4. Discussion

Histopathological alterations are considered dependable biomarkers of stress and are widely used as indices for pesticide toxicity in fish **(van der Oost *et al.,* 2003)**. Several experiments indicated that pesticides caused hepatotoxicity, haematotoxicity **(Kalender *et al.,* 2010; Lu *et al.*, 2018; Das *et al.*, 2022)**, neurotoxicity **(Das and Mukherjee, 2000)**, genotoxicity **(Giri *et al.,* 2002)** and nephrotoxicity **(Al-Attar, 2010)** in different animal models. In this study, the EB medication at the recommended dose caused mortalities and reductions in feed intake and biomass on day 21 of ED. The effect was increased proportionately in a dose-dependent manner and interfered with various organs and blood cells of *O. niloticus* like in earlier studies **(Julinta *et al.,* 2020a, 2020b; Singha *et al.,* 2022; Das *et al.,* 2022)**. Our histopathological observations further suggested a direct but dose-dependent relationship between EB medication and the functioning of various vital organs and ultimately the biosafety of *O. niloticus*. The major kidney histopathological change due to EB was renal tubular epithelial degeneration and this process led to tissue necrosis at higher doses. Tubular degeneration may occur in the form of vacuolization, the ragged appearance of the epithelial lining, atrophied epithelium and necrosis **(Heikal *et al.,* 2012)**. Tubular vacuolization and necrosis perceived in the kidneys might be the consequence of the failure of ion pump transport of tubular cells and the inability of renal cells to cope with functional disorders incited by EB. Inflammation of renal tubules observed in the present study could be ascribed to disruption of cell volume and ion homeostasis by EB, possibly by increasing ion permeability and obstructing energy production, thus leading to ATP depletion. The EB also caused fragmented and shrunken glomeruli with dilated Bowman's space in the 2.5× - 10× groups. Alike, degeneration of renal tubules, inflammation, vacuolation in renal tubular cells and widened tubular lumen were documented in different fish species subjected to varied pesticides **(Ullah and Zorriehzahra, 2015; Faheem and Lone, 2017)**. The dilation of Bowman's space suggested an increase in the filtration rate and a probable mechanism of counteracting toxicant stress. The observed mild but dose-dependent increase in the histopathological changes during the ED and PED periods possibly indicated an impairment of kidney function. The tolerability of *O. niloticus* to the recommended EB dose (1×) even during the extended dosing period was confirmed by the lack of major changes in the histological sections of the kidney. This study, in general, brought into light the renal toxicity induced by EB, which was found to be significant at higher doses. The pathological alterations observed in the kidney of *O. niloticus* could be attributed to internal exhaustion because of the interaction between EB and renal tissue. These alterations could be understood as a defence mechanism against EB and could be reversible. Contrarily, no histopathological observations were noted in EB-treated Atlantic salmon *Salmo salar* smolts **(Stone *et al.,* 2002)**.

The fish liver is the most delicate organ and hepatocyte alterations due to drugs may be useful biomarkers, as they occupy 85% of the teleost's liver volume **(van der Oost *et al.,* 2003)**. As observed in erythrocytes, stressor-associated alterations of hepatocytes may be found in the nucleus, the cytoplasm, or both **(Das *et al.,* 2022)**. In this study, the histopathology of the liver of *O. niloticus* fed the EB-diets (1× - 10×) showed mild to severe glycogen-type vacuolation, indicating the loss of cytoplasmic hepatic glycogen as an early toxic response. The hepatocytes were vacuolated and lost the usual polyhedral shape. Such direct toxic effects may disturb the detoxification or cleansing mechanisms of the liver. Besides, all EB-dosed groups documented varying degrees of cytoplasmic vacuolation and degeneration of the hepatocytes like what **Bowker *et al.* (2013)** observed in *O. mykiss* fingerling fed 150 µg/kg biomass/day or other pesticides and toxicants **(El-Murr *et al.,* 2015; Lu *et al.,* 2018)**. The existence of glycogen-type vacuolar degeneration of hepatocytes may be a consequence of extreme exertion required by the liver to get rid of EB-toxicant during the process of detoxification. Nevertheless, vacuolar degeneration is a revocable injury, and cells can revert to normal functions or homeostasis when the stress is removed **(Roberts, 2012)**. However, the recuperation of cells rests on the severity and duration of exposure to the stressors. The general feature of the liver of EB-dosed fish was the enhancement of the degree of structural heterogeneity with increasing doses. Contrarily, during the gross necropsy or histopathological examination, no pathological signs of EB-toxicity were identified in *S. salar* and *O. mykiss* (**Roy *et al.,* 2000; Stone *et al.,* 2002)**. In our previous study, the EB-dosed *O. niloticus* had significantly higher ALT and AST levels on day 7 ED than the control group **(Julinta *et al.,* 2020b; Singha *et al.,* 2022)** and these changes were consistent with the injury to the hepatic tissues of the EB-dosed groups.

In fish, the first organ to interact with medicated feeds is the intestine and the wide microvilli structure results in a large surface area that is well suited for absorption. The elements absorbed through the intestine are dispersed to the liver via the portal vein, as over 75% of the total blood flow to the liver comes from the intestines **(DeSesso and Jacobson, 2001)**. Therefore, intestinal and liver metabolism can alter the oral absorption of a drug **(Zhang and Benet, 2001)**. The control intestine was characterized by the existence of well-differentiated enterocytes with many absorptive vacuoles and goblet cells. On the other hand, all EB-dosed groups documented dose-dependent inflammation, loss of absorptive vacuoles, necrotized intestinal villi, mucinous degeneration, necrotized absorptive region and degeneration of the epithelial layer. Similarly, the intestine of *Oreochromis* spp. subjected to varied pesticides revealed mucinous degeneration, epithelial degeneration, inflammatory cell infiltration in submucosal oedema and alterations in the intestinal wall **(Soufy *et al.,* 2007; El-Murr *et al.,* 2015)**. The degenerative changes observed in the different intestinal layers may be due to a reduction in oxygen supply to the tissue **(Miller and Zachary, 2017)** and damaged erythrocytes **(Das *et al.,* 2022)**. The 5× and 10× groups recorded swollen lamina propria, which may be the direct effect of higher EB doses for a longer period, which may reduce the lamina propria facilitated contractile activity of the intestine.

The degeneration of the epithelial layer and inflammation in the intestine of EB-dosed *O. niloticus* indicated a failure in the functional integrity of the intestinal mucosal epithelial cells and the harmonized regulation of the mucus layer, the intercellular tight junction, epithelial cells and the host's immune responses **(Dharmani *et al.,* 2009)**. The histological examination also revealed necrotized intestinal villi and absorptive region, mucinous degeneration and loss of absorptive vacuoles in all EB-dosed groups, suggesting a breach of the first line of defence. The direct EB toxicity might have caused intestinal irritation and destruction of the mucous membrane. The histopathological variations observed in different intestinal layers of the present study may be due to the direct consequence of EB. The results revealed that the recommended dose of EB can cause apparent pathological changes in the intestine of *O. niloticus* during extended feeding. Though the nervous system is the target of EB toxicity **(USDA, 2010)**, the neurotoxic nature of EB was not attempted in this research. Yet our earlier study **(Singha *et al.,* 2022)** established the neurotoxic potential of EB.

5. Conclusion

Overall, the EB-dosing experiments in healthy *O. niloticus* suggested EB's hepatotoxic, nephrotoxic and intestinal toxic potential in a dose-dependent manner. Though the EB at the recommended dose and higher doses caused dose-dependent mortalities, reduced feed intake and biomass during the extended dosing, it is unlikely to develop belligerent and irrevocable effects on *O. niloticus* at the dose of 50 µg/kg biomass/day and dosage period of 7 consecutive days. The recommended dose of EB persuaded a meagre to mild histopathological changes in the kidney, liver and intestine of healthy *O. niloticus*. With the termination of medication, most of the specific changes were significantly improved, indicating the ability of fish to mount adaptive responses to recuperate. Therefore, the EB may be included in the treatment of external parasites in tropical freshwater fish to improve health and population resilience.

Acknowledgement

The authors thank the Vice-Chancellor, West Bengal University of Animal and Fishery Sciences, Kolkata, for providing the necessary facilities for carrying out this work.

Conflict of Interest

The authors report no conflicts of interest. The authors are responsible for the content of the paper.

Funding

Indian Council of Agricultural Research (ICAR), Government of India, New Delhi, under the All-India Network Project on Fish Health vide Grant F.No. CIBA/AINP-FH/2015-16 dated 02.06.2015.

Ethics approval

The current study was performed in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocols were approved by the ICAR, Government of India, New Delhi, under the All-India Network Project on Fish Health (F.No. CIBA/AINP-FH/2015-16 dated 16.7.2015)

References

1. Al-Attar, A.M., 2010. Physiological and histopathological investigations on the effects of α-lipoic acid in rats exposed to malathion. *Journal of Biomedicine and Biotechnology* 2010: 203503. https://doi.org/10.1155/2010/203503
2. Anandaraja, R., 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. https://doi.org/10.1016/j.aquaculture.2020.735335
3. Anandaraja, R, Patil, P.K., Avunje, S., Kumaran, M., Solanki, H.G., Jithendram, K.P., Alavandi, S.V., Vijayan, K.K., 2022. Efficacy of

emamectin benzoate in controlling natural infestations of ectoparasites in economically important fish species of India. *Aquaculture* 551: 737940. https://doi.org/10.1016/j.aquaculture.2022.737940

4. Anon, 2021. Major Uses of Pesticides: Insecticides. Available at: Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India, New Delhi. https://www.epestodisha.nic.in/public/documents/Major%20Use%20as%20on%201.01.2021.pdf Accessed May 10, 2022.
5. Armstrong, R., MacPhee, D., Katz, T., Endris, R., 2000. A field efficacy evaluation of emamectin benzoate for the control of sea lice in Atlantic salmon. *The Canadian Veterinary Journal* 41: 607-612.
6. AVMA, 2020. AVMA Guidelines for the euthanasia of animals, 2020 edition. American Veterinary Medical Association, Schaumburg, IL, USA. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf, Accessed May 10, 2022
7. Behera, B.K., Pradhan, P.K., Swaminathan, T.R., Sood, N., Paria, P., Das, A., Verma, D.K., Kumar, R., Yadav, M.K., Dev, A.K., Parida, P.K., Das, B.K., Lal, K.K., Jena, J.K., 2018. Emergence of Tilapia Lake Virus associated with mortalities of farmed Nile tilapia *Oreochromis niloticus* (Linnaeus 1758) in India. *Aquaculture* 484: 168–174. https://doi.org/10.1016/j.aquaculture.2017.11.025
8. Bowker, J.D., Carty, D., Bowman, M.P., 2013. The safety of SLICE (0.2% emamectin benzoate) administered in feed to fingerling rainbow trout. *North American Journal of Aquaculture* 75(4): 455-462. https://doi.org/10.1080/15222055.2013.806383
9. CPCSEA, 2021. Guidelines for experimentation on fishes. Committee for the Purpose of Control and Supervision on Experiments on Animals, Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying, Government of India. http://cpcsea.nic.in/WriteReadData/userfiles/file/GuidelinesofCPCSEAfor20 Experimentationon20Fishes-2021.pdf. Accessed May 15, 2022. 63 pp.
10. Das, B.K., Mukherjee, S.C., 2000. A histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. *Veterinarski Arhiv* 70(4): 169-180.
11. Das, R., Abraham, T.J., Singha, J., Bardhan, A., Patil, P.K., 2022. Dietary emamectin benzoate induces dose-dependent variations in haemato-biochemical and erythrocyte-metric parameters of *Oreochromis niloticus* (L.). *Aquaculture* 561: 738680. https://doi.org/10.1016/j.aquaculture.2022.738680
12. DeSesso, J.M., Jacobson, C.F., 2001. Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. *Food and Chemical Toxicology* 39(3): 209–228. https://doi.org/10.1016/S0278-6915(00)00136-8
13. Dharmani, P., Srivastava, V., Kissoon-Singh, V., Chadee, K., 2009. Role of intestinal mucins in innate host defense mechanisms against pathogens. *Journal of Innate Immunity* 1(2): 123–135. https://doi.org/10.1159/000163037
14. El-Murr, A.E., Imam, T.S., Hakim, Y., Ghonimi, W.A.M., 2015. Histopathological, immunological, hematological, and biochemical effects of fipronil on Nile tilapia (*Oreochromis niloticus*). *Journal of Veterinary Science and Technology* 6: 252, http://dx.doi.org/10.4172/2157-7579.1000252
15. Faheem, M., Lone, K.P., 2017. Oxidative stress and histopathologic biomarkers of exposure to bisphenol-A in the freshwater fish, *Ctenopharyngodon idella. Brazilian Journal of Pharmaceutical Sciences* 53(*3*): e17003. https://doi.org/10.1590/s2175-97902017000317003
16. FAO, 2020. The state of world fisheries and aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en
17. Giri, S., Prasad, S.B., Giri, A., Sharma, G.D., 2002. Genotoxic effects of malathion an organophosphorus insecticide, using three mammalian bioassays in vivo. *Mutation Research* 514(1-2): 223–231. https://doi.org/10.1016/S1383-5718(01)00341-2
18. González-Gaya, B., García-Bueno, N., Buelow, E., Marin, A., Rico, A., 2022. Effects of aquaculture waste feeds and antibiotics on marine benthic ecosystems in the Mediterranean Sea. *Science of the Total Environment* 806(2022): 151190. https://doi.org/10.1016/j.scitotenv.2021.151190
19. Heikal, T.M., Mossa, A.T.H., Marci, G.I., Rasoul, M.A., 2012. Cyromazine and chlorpyrifos induced renal toxicity in rats: The ameliorating effects of green tea extract. *Environmental and Analytical Toxicology* 2(*5*): 146.
20. Jansen, M.D., Dong, H.T., Mohan, C.V., 2018. Tilapia lake virus: a threat to the global tilapia industry?. *Reviews in Aquaculture* 11(*3*): 725-739. https://doi.org/10.1111/raq.12254
21. Julinta, R.B., Abraham, T.J., Roy, A., Singha, J., Bardhan, A., Sar, T.K., Patil, P.K., Kumar, K.A., 2020a. Safety of emamectin benzoate administered in feed to Nile tilapia *Oreochromis niloticus* (L.). *Environmental Toxicology and Pharmacology* 75: 103348. https://doi.org/10.1016/j.etap.2020.103348
22. Julinta, R.B., Abraham, T.J., Roy, A., Singha, J., Dash, G., Sar, T.K., Patil, P.K., 2020b. Effects of oral-dosing of an antiparasitic drug emamectin benzoate on the growth and serum biomarkers of *Oreochromis niloticus* (L.) juveniles. *Journal of Environmental Biology* 41(5): 973-979. http://dx.doi.org/10.22438/jeb/41/5/MRN-1222
23. Kalender, S., Uzun, F.G. Durak, D., Demir, F., Kalender, Y., 2010. Malathion induced hepatotoxicity in rats: the effects of vitamins C and E. *Food and Chemical Toxicology* 48(*2*): 633-638. https://doi.org/10.1016/j.fct.2009.11.044
24. Kumar, G., Gaunt, P., 2020. Medicated-feed intervention in catfish farming: An economic perspective. *North American Journal of Aquaculture* 82(2): 190-199. https://doi.org/10.1002/naaq.10143
25. Lees, F., Baillie, M., Gettinby, G., Revie, C.W., 2008. The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L) in Scotland, 2002–2006. *PLoS One* 3(2): e1549. https://doi.org/10.1371/journal.pone.0001549
26. Lu, J., Zhang, M., Lu, L., 2018. Tissue metabolism, hematotoxicity and hepatotoxicity of trichlorfon in *Carassius auratus gibelio* after a single oral administration. *Frontiers in Physiology* 9: 551. https://doi.org/10.3389/fphys. 2018.00551
27. Miller, M.A., Zachary, J.F., 2017. Mechanisms and morphology of cellular injury, adaptation, and death. Pathologic Basis of Veterinary Disease 2–43: e19. DOI: https://doi.org/10.1016/B978-0-323-35775-3.00001-1
28. Moschou, I.C., Dasenaki, M.E., Thomaidis, N.S., 2019. Ionization study and simultaneous determination of avermectins and milbemycines in fish tissue by LC-ESI-MS/MS. *Journal of Chromatography* B 1104: 134-140. https://doi.org/10.1016/j.jchromb. 2018.11.017
29. MSD Animal Health, 2012. SLICE technical monograph. Merck Animal Health, Whitehouse Station, New Jersey. https://www.msd-animal-health.no/wpcontent/uploads/sites/15/2020/03/Slice-SSP-Monograph_tcm84-151889.pdf . Accessed May 10, 2022, 36pp.
30. Nilsen, A., Nielsen, K.V., Biering, E., Bergheim, A., 2017. Effective protection against sea lice during the production of Atlantic salmon in floating enclosures. *Aquaculture* 466: 41-50. https://doi.org/10.1016/j.aquaculture.2016.09.009
31. Patil, P.K., Mishra, S.S., Pradhan, P.K., Manna, S.K., Abraham, T.J., Solanki, H.G., et al., 2022. Usage pattern of chemicals, biologicals, and veterinary medicinal products in Indian aquaculture. *Reviews in Aquaculture* 14(4): 2038-2063. https://doi.org/10.1111/raq.12688
32. Powell, A., Treasurer, J.W., Pooley, C.L., Keay, A.J., Lloyd, R., Imsland, A.J., de Leaniz, C.G., 2018. Use of lumpfish for sea-lice control in salmon farming: Challenges and opportunities. *Reviews in Aquaculture* 10(3): 683-702. https://doi.org/10.1111/raq. 12194
33. Prabu, E., Rajagopalsamy, C.B.T., Ahilan, B., Jeevagan, I.J.M.A., Renuhadevi, M., 2019. Tilapia–an excellent candidate species for world aquaculture: a review. *Annual Research and Review in Biology* 31(3): 1-14. https://doi.org/10.9734/arrb/2019/v31i330052
34. Roberts, R.J., 2012. Fish pathology, 4th edn. Wiley-Blackwell, UK. 581 pp.
35. Roy, W.J., Sutherland, I.H., Roger, H.D.M., Varma, K.J., 2000. Tolerance of Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. *Aquaculture* 184: 19-29. https://doi.org/10.1016/S0044-8486(99)00307-5
36. Sahoo, P.K., Mohanty, J., Garnayak, S.K., Mohanty, B.R., Kar, B., Prasanth, H., Jena, J.K., 2013. Estimation of loss due to argulosis in carp culture ponds in India. *Indian Journal of Fisheries* 60: 99-102.
37. Sanders, P., Swan, G., 2014. Emamectin benzoate. In: *Joint FAO/WHO Expert Committee on Food Additives.* Food and Agriculture Organization of the United Nations, Rome, pp: 23-37.
38. Singha, J., Abraham, T.J., Roy, A., Bardhan, A., Sar, T.K., Rajisha, R., Krishna, E.K.N., Kumar, K.A., Patil, P.K., 2022. Influence of dietary emamectin benzoate on the biological responses of monosex (all-male) *Oreochromis niloticus* (L.) fries. *Comparative Biochemistry and Physiology Part-C Toxicology and Pharmacology* 252: 109223. https://doi.org/10.1016/j.cbpc.2021.109223

39. Soufy, H., Soliman, M.K., El-Manakhly, E.M., Gaafar, A.Y., 2007. Some biochemical and pathological investigations on monosex tilapia following chronic exposure to carbofuran pesticides. *Global Veterinaria* 1(1): 45-52.
40. St-Hilaire, S., Cheng, T.H., Chan, S.C.H., Leung, C.F., Chan, K.M., Lim, K.Z., Furtado, W., Gomes, G.B., 2021. Emamectin benzoate treatment of hybrid grouper infected with sea lice in Hong Kong. *Frontiers in Veterinary Science* 8: 646652. https://doi.org/10.3389/fvets.2021.646652
41. Stone, J., Roy, W.J., Sutherland, I.H., Ferguson, H.W., Sommerville, C., Endris, R., 2002. Safety and efficacy of emamectin benzoate administered in-feed to Atlantic salmon *Salmo salar* L., smolts in freshwater, as a preventative treatment against infestations of sea lice, *Lepeophtheirus salmonis* (Kroyer). *Aquaculture* 210: 21-34. https://doi.org/10.1016/S0044-8486(01)00822-5
42. Ullah, S., Zorriehzahra, M.J., 2015. Ecotoxicology: a review of pesticides induced toxicity in fish. *Advances in Animal and Veterinary Sciences* 3(1): 40-57. https://doi.org/10.14737/journal.aavs/2015/3.1.40.57
43. USDA, 2010. Emamectin benzoate human health and ecological risk assessment final report. United States Department of Agriculture. http://www.fs.fed.us/foresthealth/pesticide/pdfs/052-23-03b_Emamectin-benzoate.pdf . Accessed May 10, 2022
44. Zamri-Saad, M., Amal, M.N.A., Siti-Zahrah, A., Zulkafli, A.R., 2014. Control and prevention of streptococcosis in cultured tilapia in Malaysia: a review. *Pertanika Journal of Tropical Agricultural Science* 37(4): 389-410.
45. Zhang, Y., Benet, L.Z., 2001. The gut as a barrier to drug absorption combined role of cytochrome PCYP 3A and p-glycoprotein. *Clinical Pharmacokinetics* 40(3): 159–168. d https://doi.org/10.2165/00003088-200140030-00002