American Journal of Pharmacology and Toxicology

Research Article

# Effects of Dietary Emamectin Benzoate at Recommended and Overdose Levels on the Pathophysiological Responses and Tolerability of *Oreochromis niloticus* at Fry Stage

**Jasmine Singha[1], Thangapalam Jawahar Abraham[1], Satyen Kumar Panda[2] and Prasanna Kumar Patil[3]**

[1]*Department of Aquatic Animal Health, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata, India*
[2]*Fish Processing Division, ICAR-Central Institute of Fisheries Technology, Willington Island, Kochi, India*
[3]*Aquatic Animal Health and Environment Division, ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India*

*Article history*
Received: 11-03-2026
Revised: 17-04-2026
Accepted: 25-04-2026

**Corresponding Author:**
Thangapalam Jawahar Abraham
Department of Aquatic Animal Health, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata, India
Email: abrahamtj1@gmail.com

**Abstract:** Emamectin Benzoate (EB) has been recommended as a feed additive for the control of fish ectoparasites. This investigation examined the impact of dietary EB on physiological responses, antioxidant defence mechanisms, and histopathological changes in key organs, and on the kinetics of residue deposition and elimination in the muscle of *Oreochromis niloticus* fry. Fry were administered EB feeds at 50 µg (recommended dose) and 500 µg/kg biomass/day (overdose) for 7 days and observed for 42 days post-EB-dosing. The reductions in superoxide dismutase, calcium, chloride, and acetylcholinesterase and the elevations in glucose and alkaline phosphatase, indicated failure in the antioxidant capacity, ionic equilibrium, brain and liver functions, and physiological responses. The dose-dependent mortalities and biomass of fry were recorded. Histopathological alterations in the livers, kidneys, intestines, and brains indicated the toxicity of EB. The brain tissue exhibited a reduction in neuron density, with remaining cells appearing scattered. Upon cessation of medication, the physiological responses of the fry were normalised. The EB residues peaked on day 7 (4.18 ng/g) and decreased significantly to trace levels on day 42 post-dosing (0.12 ng/g) in the recommended dose group. The muscle EB residue accumulation was within the permissible maximum residue limits (100 ng/g) set by international agencies. The findings on pharmacological behaviour and multiple biomarkers suggested that EB at the recommended dose is convincingly safe for *O. niloticus* fry.

**Keywords:** Tilapia Aquaculture, Antiparasitic Veterinary Drug, Drug Residues, Oxidative Stress, Histoarchitecture

## Introduction

Global aquatic animal production rose to 185 million tons in 2022, a 4% increase from 2020. Of this total, around 115 million tons originated from marine environments and 70 million tons from inland systems. Cichlids are the third most widely cultivated group in global aquaculture, after carps and catfish. Within this group, Nile tilapia (*Oreochromis niloticus*) alone accounted for 5.3 million tons in 2022 (FAO, 2024). As aquaculture has become more intensive, fish are increasingly prone to microbial infections, resulting in significant economic losses (Patil et al., 2025). Globally, veterinary medicinal products (VMPs) have been used consistently to maintain healthy harvests and avoid production losses in aquaculture (Padros et al., 2024; Rico et al., 2013). Reports on Indian aquaculture practices highlighted a heavy reliance on antimicrobials, with evidence indicating that around 109 commercial formulations are used to manage disease outbreaks (Patil et al., 2022; Singha et al., 2021). The intensive aquaculture production and the growing use of VMPs have raised concerns about environmental health, adverse impacts on non-targeted aquatic organisms, and the potential for residue accumulation in farmed fish, which could pose risks to consumers (Rico et al., 2019). To address this issue, specific withdrawal periods have been set to indicate the minimum time between the last drug dose and fish consumption (Limbu et al., 2021). Emamectin Benzoate (EB), an avermectin compound

produced by the bacterium *Streptomyces avermitilis*, was created as an agricultural pesticide. It is currently used in aquaculture as a medicated feed additive to manage sea-lice infestations in farmed fish across tropical and temperate regions, typically at 50 µg/kg biomass/day for 7 days (Anandaraja et al., 2022; 2020; St-Hilaire et al., 2021). Management practices associated with approved VMPs by the United States Food and Drug Administration (USFDA) may increase the risk of antimicrobial resistance, particularly through overuse and inappropriate dosing regimens (USFDA, 2014).

Orally administered EB exerts neurotoxic effects by selectively interacting with glutamate-gated chloride channels within neuronal and muscular tissues, thereby disrupting metabolic processes in fish (Singha et al., 2022; Julinta et al., 2020a-b). According to Kennedy et al. (2014), pesticides can compromise the Blood-Brain Barrier (BBB) and induce neurotoxic effects that inhibit acetylcholinesterase (AChE) production in the synaptic region of the neurotransmission pathway, thereby suggesting a suitable fish biomarker that can disrupt enzymatic production even at trace levels. Furthermore, the avermectin group has been shown to induce neuronal damage, which correlates with decreased body antioxidant capacity due to oxidative stress (Zhang et al., 2022). In addition, the changes in biochemical enzymes can lead to alterations at the cellular and tissue levels. EB residues can persist in sediments for a significant duration, with a half-life exceeding 120 days (Kumar et al., 2022). In tropical aquaculture systems, limited information on EB application and the absence of robust scientific guidance create uncertainty about the safety of EB-treated farmed fish for human consumers. Our previous studies examined the biosafety of EB in *O. niloticus* fish at two life stages (juveniles and fry) at recommended and overdose levels, when administered over extended feeding periods (Abraham et al., 2024, 2022; Singha et al., 2022; Julinta et al., 2020a-b). This study sought to assess the pharmacological behaviour of EB in muscle tissue during the 7-day recommended dosing phase and the 42-day monitoring period, and alterations in the biochemical, histopathological, and antioxidant capacity of all-male *O. niloticus* at the fry stage under tropical Indian conditions. In addition, the tolerability of fry was evaluated under overdose conditions, i.e., at ten times the recommended dose.

## Materials and Methods

### *Animal Ethical Compliance*

This research was conducted in compliance with the fish experimentation guidelines as prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2021), Ministry of Fisheries, Animal Husbandry, and Dairying, Government of India, New Delhi. The experimental procedures received ethical clearance from the Indian Council of Agricultural Research (ICAR), Government of India, New Delhi, under the All-India Network Project on Fish Health (F. No. CIBA/AINP-FH/2015-16; dt 16.07.2015).

### *Acclimatization and Experimental Design*

Healthy and active, *Oreochromis niloticus* fry (all-male; 0.46 ± 0.05 g), reared under hatchery conditions, were sourced from a commercial facility. Within 3 hours of collection, the fry were transferred to the laboratory with proper oxygen packing. Upon arrival, the fry were allowed to settle for 2 hours, after which they were gently disinfected for 2-3 min in a 2-ppm potassium permanganate bath, shifted to circular holding tanks of 500-L capacity at a density of 400 fry/tank, and allowed to acclimate for 15 days under constant aeration. Fry were fed with a high-quality commercial floating pellet diet (Aquaxcel, Cargill, India) according to the feeding practices described in earlier investigations (Singha et al., 2022; Singha and Abraham, 2025). The acclimatised fry (initial mean bodyweight (BW): 0.63 ± 0.03 g) without any abnormalities or signs of infection were randomly selected and transferred into circular tanks (Number of tanks = 9; 220 fry/tank) containing 450 L of water, and fed as above (feeding rate: 6% BW/day). Approximately 100 L of water was replaced every other day to clear accumulated waste and uneaten feed. The fry were distributed to 3 treatment sets:

(i) Control (0×)
(ii) Recommended dose (1×; 50 µg)
(iii) Overdose (10×; 500 µg), with each treatment maintained in triplicate

Water quality parameters, such as temperature (23.04-29.00°C), pH (7.60-8.20), dissolved oxygen (4.22-5.61 ppm), nitrate (0.27-0.48 ppm), nitrite (0.12-0.27 ppm), and ammonia (0.002-0.007 ppm), were maintained within optimal ranges in each treatment tank. The photoperiod during the experimental period was 13.1-13.5 (light): 10.5-10.9 (dark) hours

### *EB Feed Preparation*

Based on the biomass, the amount of EB added to the feed was calculated to ensure the fish received the intended daily recommended dose (50 µg) and an overdose (500 µg) to assess the safety and tolerability of the fry. The top-coated EB diets were prepared as described in our previous studies (Singha et al., 2022; Julinta et al., 2020a). Briefly, the required amount of EB powder (Sigma-Aldrich, India, CAS number 155569-91-8; ≥85% purity) was emulsified in 10 mL vegetable oil and then admixed with a kg of basal pellet feed.

### *EB Dose Administration*

The experiment consisted of four phases: pre-dosing (0-7 days), fasting (day 8), a 7-day EB-dosing period (ED; 9-15 days), and a 42-day post-dosing phase (PED; 16-57 days), as designed previously (Singha and Abraham, 2025). To improve treatment efficacy, the fry were fasted for a day before dosing to empty their gastrointestinal tract and absorb EB more effectively. All groups initially received the control diet before dosing to maintain continuous feeding to satiety. The two treatment groups were then provided the respective EB diets for 7 days, after which they returned to the control diet for the remainder of the trial. Fry were provided feed equivalent to 6% BW/day, distributed in three equal portions at 4-hour intervals commencing at 08:00 in the morning. Daily observations included water-column position, respiratory distress, abnormal swimming, pigmentation, behavioural deviations, and mortality. Uneaten feed was recovered 75 minutes after each meal, dried under the fan for 24 h at room temperature, and quantified tank-wise. Weekly biomass measurements of at least 10 fry/tank were used to adjust ration size. Feeding responses were classified using an ordinal scoring system, as described by Bowker et al. (2013).

### *Collection of Muscle and Brain Tissues and Determination of Clinical Biochemistry*

Three fry/tank were sampled at different time points, i.e., days 0 and 7 of the ED phase, and days 28 and 42 of the PED phase, and euthanised with eugenol (20 µL/L). Following dissection, degutting and rinsing, the required tissues were isolated. Muscle samples were collected after removing the skin, and whole brains were excised with care to avoid contamination with blood or non-neural material. All tissues were stored at −20°C. Tank-wise pooled muscle samples were later thawed and processed according to the description of Singha et al. (2022). To determine physiological responses, selected biochemical markers were analysed, as described in Singha et al. (2022). Measurements included glucose, calcium, Alkaline Phosphatase (ALP), Superoxide Dismutase (SOD), and AChE activity in the targeted tissues, and were quantified using validated commercial kits. The SOD assay kit was obtained from Cayman Chemical, United States of America, while other clinical chemistry assay kits were sourced from DiaSys Diagnostic Systems, Germany.

### *Histopathology*

Two fry/tank were randomly collected at different time points, i.e., days 0 and 7 of the ED phase, and day 42 of the PED phase, euthanised, and the targeted organs, such as liver, kidney, intestine, and brain, were isolated carefully. Bouin solution fixed samples were processed, sectioned, stained, and examined (Roberts, 2012), and images were captured as described in Singha and Abraham (2025). The key tissue alterations were identified and evaluated using a 0-5 ordinal scoring scale based on the proportion of tissue alteration from its normal structure (Bowker et al. 2013).

### *Uptake and Depletion of EB Residues*

Healthy fry (n = 10 each) were sampled on days 0, 1, 3, and 7 of the ED phase, and on days 7, 14, 21, 35, and 42 of the PED phase from the treated and control tanks. Fry were euthanised as described earlier, and muscle tissues were collected for residue analysis. Extraction, cleanup, and quantification of EB residues in the muscle tissues were carried out by LC-MS/MS as elaborated earlier (Singha et al., 2022; Julinta et al., 2020a). Method validation covering all requisite parameters was achieved in accordance with established governing guidelines (SANCO, 2012; European Commission, 2002; 2021). The calculation of the withdrawal period of EB followed the European Medicines Agency (EMA, 2018).

### *Statistical Analysis*

The adequacy of the sample size was evaluated by a post-hoc power analysis using G*Power (version 3.1.9.7). Power was estimated for both dose-wise and time-wise comparisons based on the observed effect sizes at $\alpha = 0.05$. The feed intake and qualitative scores of histopathological changes were assessed by a nonparametric Kruskal–Wallis test with pair-wise comparisons. The results of mortality, biomass, and biochemical response variables were compared among treatments, both dose- and time-wise, using one-way ANOVA, followed by Tukey's HSD for pairwise comparisons. The SPSS (Version 22.0) software performed all analyses and statistical interpretations at a significance of $P<0.05$.

## Results

### *Feed Consumption, Mortalities, and Biomass*

A consistent display of vigorous, assertive feeding behaviour was observed in the control group throughout the study period. Active foraging of feeds was lacking in the recommended dose (1×) group, which was not observed in the overdose (10×) group. Feed intake declined markedly, reaching 89.25% in the 1× group and 71.50% in the 10× group ($P<0.05$). During the PED phase, feed intake in the 1× group did not differ significantly, whereas the 10× group exhibited a pronounced reduction (Table 1). Impaired balance, reduced activity, darker pigmentation, diminished feeding response, and surface air-gasping were evident in the 10× group. Internally, affected fry showed splenomegaly, hepatomegaly, pale and swollen kidneys, gill reddening, and intestinal discolouration. The 7 days of ED documented 0.30% and 3.18% mortalities in the 1× and 10× groups (Fig. 1A), respectively, with no significant difference ($P>0.05$). An increase in mortality to 5.23% was noted in the 10× group, with a significant decrease ($P<0.05$) in biomass on day 42 PED (Fig. 1B).

**Table 1:** Qualitative assessment of feed intake in emamectin benzoate (EB)-dosed *Oreochromis niloticus* fry at 50 µg/kg biomass/day (1×) and 500 µg/kg biomass/day (10×) for 7 days and observed for 42 days post-EB-dosing

| | EB dose and qualitative scores of feed intake | | |
|---|---|---|---|
| Treatment period* | 0× | 1× | 10× |
| Pre-dosing (0-7 days) | $4.00\pm0.00^{a1}$ | $4.00\pm0.00^{a1}$ | $4.00\pm0.00^{a1}$ |
| EB-dosing (9-15 days) | $4.00\pm0.00^{a1}$ | $3.56\pm0.52^{b2}$ | $2.84\pm0.37^{b3}$ |
| Post-EB-dosing (16-57 days) | $4.00\pm0.00^{a1}$ | $4.00\pm0.00^{a1}$ | $3.86\pm0.04^{c2}$ |

The fry were starved on day 8; Within a column for a given dose, values sharing the same alphabetical superscript (a–c) did not differ significantly (P>0.05). Within a row for a specific exposure period, values sharing the same numerical superscript (1–3) did not differ significantly (P>0.05). Feed intake was scored on a scale: [0] zero feed intake, no feeding response; [1] ~25% consumed, low interest; [2] ~50% consumed, moderate interest; [3] ~75% consumed, fair interest; [4] 100% consumed, vigorous feeding

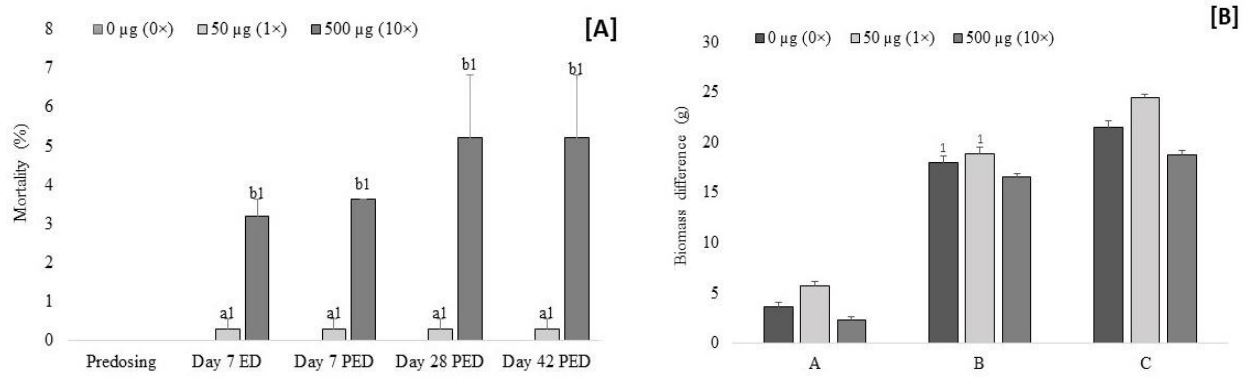


**Fig. 1:** Effects of emamectin benzoate (EB) administration on the [A] mortalities and [B] differences in biomass of *Oreochromis niloticus* fry at 50 µg and 500 µg/kg biomass/day for 7 days and observed for 42 post-EB dosing days (PED). Pre-dosing (0-7 days), a 7-day EB-dosing period (ED; 9-15 days), and a 42-day post-dosing phase (16-57 days). A = Transition point from the ED phase to the experimental period; B = Transition point from the end of the experiment to the conclusion of the ED phase; C = Entire duration of the study (beginning to end). Biomass represents measurements from 10 fry/replicate tank. Vertical bars with the same alphabetical superscript (a–b) for a given dose did not differ significantly (P>0.05). Bars sharing the same numerical superscript (1) within a dosing period were also not significantly different (P>0.05)

### *Biochemical Responses*

The concentrations of glucose increased significantly (P<0.05), peaking in the 10× group. The levels of glucose declined significantly (P<0.05) in the 1× group to normal levels by day 42 of the PED phase. In contrast, in the 10× group, hyperglycemia persisted (Fig. 2A). An insignificant reduction in calcium was recorded with dosing compared to the control (P>0.05), and the rate of reduction was higher in the 10× group. With the suspension of ED, the calcium levels increased slightly but insignificantly (P>0.05) in both groups (Fig. 2B). Similarly, chloride content in the muscle displayed the same overall pattern in all the groups (Fig. 2C). During the ED period, ALP levels increased marginally at the 1× group but surged markedly in the 10× group. On day 42 of the PED phase, both groups showed a significant reduction (P<0.05) and became almost normal in the 1× group (Fig. 2D). The stable SOD activity was significantly diminished in 7 days of ED in the 1× group and declined to almost nil in the 10× group (P<0.05). Levels increased after cessation of dosing but did not return to normal in either group (Fig. 2E). Within 7 days of ED, the AChE was reduced significantly (P<0.05), showing the highest decline in the 10× group. Though a significant increase in AChE levels was noted on day 42 of the PED phase (P<0.05), normalcy was noted only in the 1× group (Fig. 2F). The variability within the group was negligible across all sampling points. Post hoc power analysis indicated that the sample size provided high statistical power ($1 - \beta \geq 0.95$) for most biochemical parameters (glucose, calcium, ALP, and AChE) in both time- and dose-wise comparisons. However, the variables chloride and SOD exhibited comparatively lower power ($1 - \beta < 0.95$), likely reflecting smaller effect sizes or reduced variability among groups.

### *Histopathological Changes in the Liver, Kidney, Intestine, and Brain*

The predominant alteration observed in the fry livers was the presence of glycogen-type vacuolation. In both groups, histopathological features included cytoplasmic vacuolation, cellular degeneration, and focal necrosis (Figs. 3A-C). The liver parenchyma reached near-normal levels with regeneration of the portal tract and central vein in the 1× group on day 42 PED, but with a continued presence of mild glycogen-type vacuolation (Fig. 3D).

**DOI:** 10.3844/ajptsp.2026.21.34

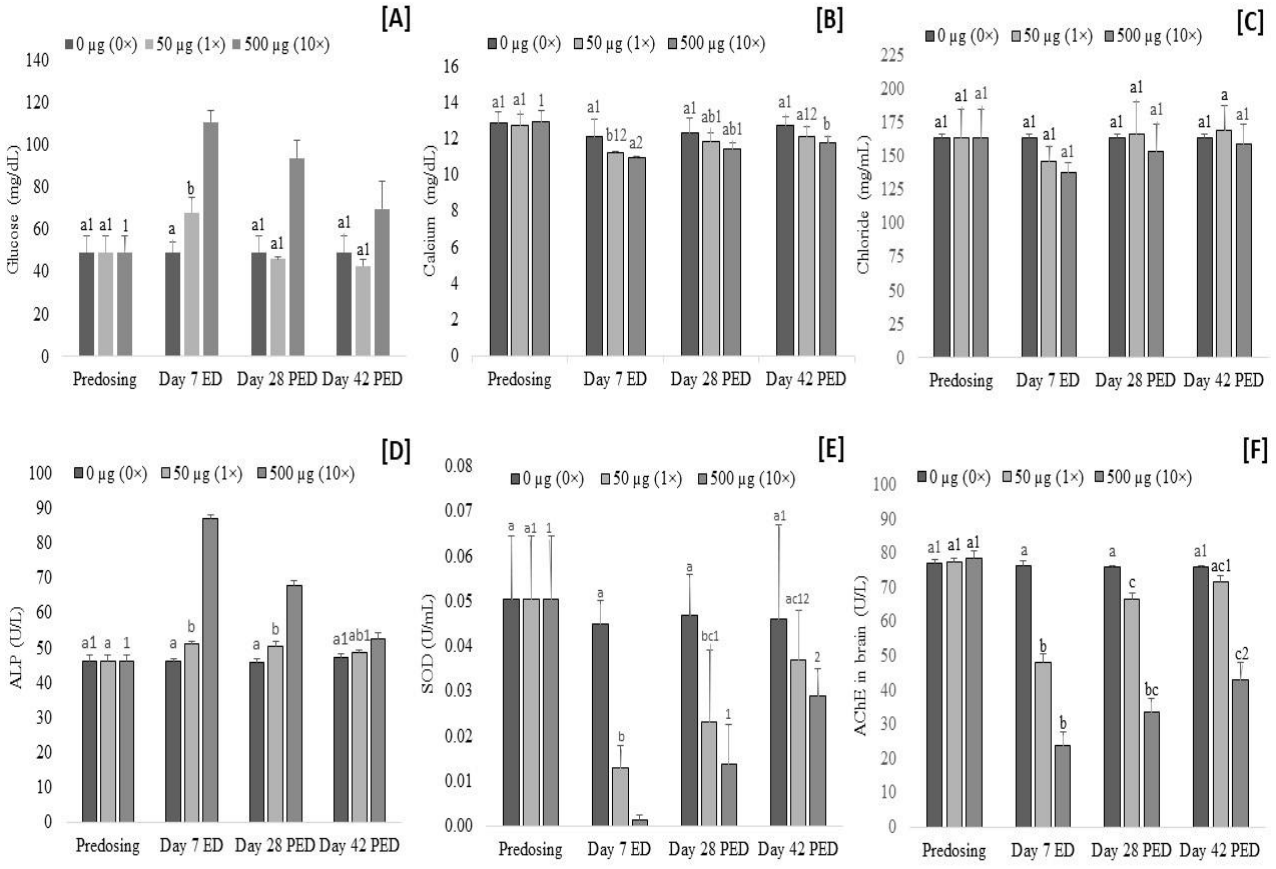


**Fig. 2:** Effects of EB administration on the parameters such as [A] glucose, [B] calcium, [C] chloride, [D] ALP, and [E] SOD in the edible muscle tissues and [F] AChE levels in the brain tissues of *Oreochromis niloticus* fry at 50 µg and 500 µg/kg biomass/day for 7 days. Pre-dosing (0-7 days), a 7-day EB-dosing period (ED; 9-15 days), and a 42-day post-dosing phase (PED; 16-57 days). Vertical bars with the same alphabetical superscript (a–c) for a given dose did not differ significantly (P>0.05). Bars sharing the same numerical superscript (1) within a dosing period were also not significantly different (P>0.05)

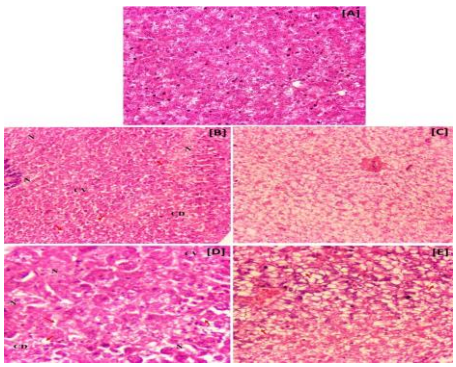

**Fig. 3:** Microscopic pathological alterations in the liver of [A] control and [B] EB-treated *Oreochromis niloticus* fry at 50 µg/kg biomass/day on day 7 of dosing and [C] day 42 post-EB-dosing; and [D] at 500 µg/kg biomass/day on day 7 of dosing; and [E] day 42 post-EB-dosing, displaying cytoplasmic degeneration (CD), cytoplasmic vacuolation (CV), necrosis (N), and glycogen-type vacuolation (red arrow). ×200; H&E staining

In the 10× group, the changes like cytoplasmic vacuolation, degeneration and glycogen-type vacuolation persisted (Fig. 3E). Changes in the fry kidneys of the 1× dose were evident throughout the study period, characterised by degeneration of the renal tubular epithelium, tubular disruption, cytoplasmic vacuolation, fragmented glomeruli, mineral deposits, and focal necrotic lesions (Figs. 4A, B). Similar observations were found in the 10× group with higher intensity and a mild necrotised hematopoietic area (Fig. 4C). The level of kidney damage was reduced during the PED phase in all the groups (Figs. 4D, E). The prominent intestinal changes were degeneration of the epithelial lining, mucinous degeneration, and disappearance of absorptive vacuolar structures. Besides, widespread necrosis affected the villi and goblet cells, alongside a compromised necrotised absorptive area (Figs. 5A-C). The degree of damage was reduced during the PED phase. However, swelling of the lamina propria was observed even on day

**DOI:** 10.3844/ajptsp.2026.21.34

42 PED (Figs. 5D, E). The observed alterations in brain tissue included degeneration of the granular layer and necrotised areas within the stratum griseum superficiale, stratum griserum centrale, and stratum album centrale, along with degeneration of the stratum opticum and stratum marginale, in both groups on day 7 of ED. These lesions were very minimal in the 1× group and minimal to mild in the 10× group (Figs. 6A-C). In the 1× group, degeneration of neuronal cells was observed, with a slightly stretched nucleus (Fig. 6B). The 10× group had pyknotic nuclei at the end of dosing (Fig. 6C). Also, there was a decrease in neuron cell numbers in the 10× group (Fig. 6C). On day 42 PED, the changes in the brain histoarchitecture were relatively improved, with the proliferation of neuron cells and recovery in the morphology of damaged nucleus in the 1× group (Fig. 6D) compared to the 10× group (Fig. 6E). The qualitative scores on the extent of damage and pathological alterations in various organs of EB-dosed fry are presented in Table 2. Overall, these changes were very minimal to mild.

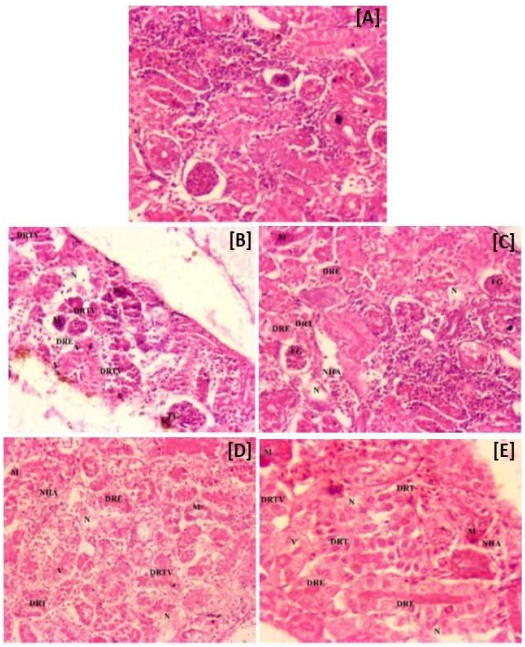


**Fig. 4:** Microscopic pathological alterations in the kidney of [A] control and [B] EB-treated *Oreochromis niloticus* fry at 50 µg/kg biomass/day on day 7 of dosing and [C] day 42 post-EB-Dosing; and [D] at 500 µg/kg biomass/day on day 7 of dosing; and [E] day 42 post-EB-dosing; displaying Degeneration of Renal tubular Epithelium (DRE), fragmented glomerulus (FG), Degeneration of Renal Tubule and Vacuolation (DRTV), Necrosis (N), vacuolation in the renal tubule (V), and mineralization in the renal tubule (M), Degeneration of Renal Tubule (DRT), and Necrotized Hematopoietic Area (NHA). ×200; H&E staining

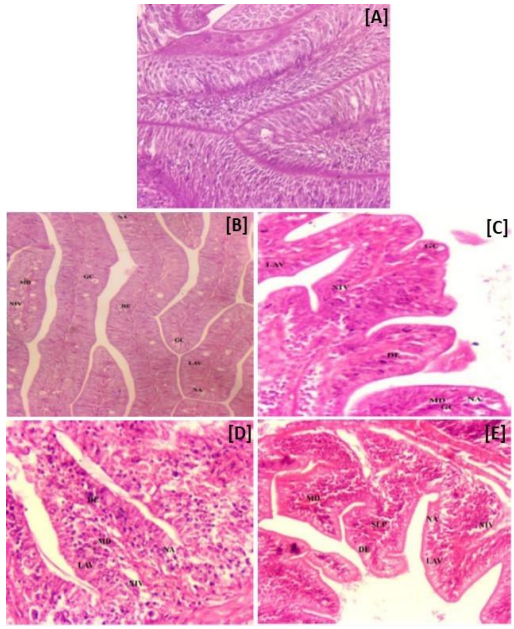


**Fig. 5:** Microscopic pathological alterations in the intestine of [A] control and [B] EB-treated *Oreochromis niloticus* fry at 50 µg/kg biomass/day on day 7 of dosing and [C] day 42 post-EB-dosing; and [D] at 500 µg/kg biomass/day on day 7 of dosing; and [E] day 42 post-EB-dosing; displaying Loss of Absorptive Vacuoles (LAV), Mucinous Degeneration (MD), Degeneration of Epithelial layer (DE), necrosis in the intestinal villi (NIV), and necrotized absorptive area (NA), goblet cells (GC), and Swelling of Lamina Propria (SLP). ×200; H&E staining

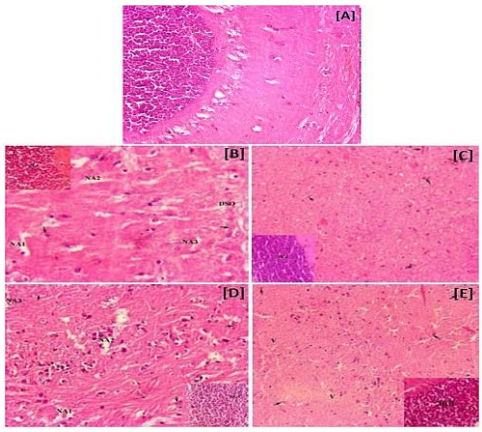


**Fig. 6:** Microscopic pathological alterations in the brain of [A] control and [B] EB-treated *Oreochromis niloticus* fry at 50 µg/kg biomass/day on day 7 of dosing and [C] day 42 post-EB-dosing; and [D] at 500 µg/kg biomass/day on day 7 of dosing; and [E] day 42 post-EB-dosing; displaying degeneration of granular layer (DGL), a necrotized area in the stratum album centrale (NA1), stratum griserum centrale (NA2), and stratum griseum superficiale (NA3), degeneration in tratum opticum (DSO), vacuolation (V) and neuron cells (black arrow). ×200; H&E staining. Superimposed images of the respective sections are the granular layer with degeneration

**Table 2:** Qualitative evaluation of the key histopathological variations in *Oreochromis niloticus* fed emamectin benzoate (EB) at 50 and 500 µg/kg biomass/day for 7 consecutive days and observed for 42 days post-EB-dosing in comparison with the control feed-fed group

| Tissue-level alterations | Qualitative scores* | | | |
|---|---|---|---|---|
| | 50 µg (1×) | | 500 µg (10×) | |
| | Day 7 ED | Day 42 PED | Day 7 ED | Day 42 PED |
| **Liver** | | | | |
| Glycogen-type vacuolation | 1.43±0.15$^{A1}$ | 2.68±0.08$^{a2}$ | 1.26±0.19$^{A1}$ | 2.51±0.11$^{a2}$ |
| Cytoplasmic vacuolation | 0.69±0.05$^{A1}$ | 0.62±0.10$^{a1}$ | 0.68±0.12 $^{A1}$ | 0.55±0.10$^{a1}$ |
| Cytoplasmic degeneration | 0.63±0.08$^{A1}$ | 0.51±0.08$^{a1}$ | 0.93±0.12$^{B1}$ | 0.45±0.10$^{a2}$ |
| Necrosis | 0.88±0.11$^{A1}$ | 0.38±0.04$^{a2}$ | 1.18±0.20$^{B1}$ | 0.53±0.08$^{b2}$ |
| **Kidney** | | | | |
| Degeneration of renal tubules and tubular epithelium | 2.13±0.12$^{A1}$ | 1.52±0.17$^{a2}$ | 2.57±0.21$^{A1}$ | 1.74±0.12$^{a2}$ |
| Vacuolation in the renal tubule | 1.13±0.20$^{A1}$ | 0.83±0.16$^{a1}$ | 1.63±0.16$^{B1}$ | 0.99±0.07$^{a2}$ |
| Necrosis | 1.88±0.17$^{A1}$ | 1.18±0.12$^{a2}$ | 2.47±0.19$^{B1}$ | 1.25±0.14$^{a2}$ |
| Glomerulopathy# | 0.96±0.05$^{A1}$ | 0.53±0.08$^{a2}$ | 1.43±0.13$^{B1}$ | 0.58±0.08$^{a2}$ |
| Mineralization | 1.18±0.19$^{A1}$ | 0.83±0.08$^{a2}$ | 1.20±0.07$^{A1}$ | 0.69±0.07$^{a2}$ |
| **Intestine** | | | | |
| Loss of absorptive vacuoles | 2.47±0.22$^{A1}$ | 1.77±0.12$^{a2}$ | 2.65±0.15$^{A1}$ | 1.28±0.06$^{b2}$ |
| Mucinous degeneration | 1.01±0.14$^{A1}$ | 0.30±0.10$^{a2}$ | 1.22±0.05$^{B1}$ | 0.68±0.10$^{b2}$ |
| Degeneration of the epithelial layer | 0.30±0.05$^{A1}$ | 0.28±0.04$^{a1}$ | 0.31±0.07$^{A1}$ | 0.23±0.08$^{a1}$ |
| Necrosis of the absorptive region | 0.48±0.08$^{A1}$ | 0.37±0.08$^{a1}$ | 0.50±0.06$^{B1}$ | 0.36±0.07$^{a2}$ |
| Necrosis of intestinal villi | 0.44±0.11$^{A1}$ | 0.42±0.08$^{a1}$ | 0.47±0.08$^{A1}$ | 0.41±0.11$^{a1}$ |
| **Brain** | | | | |
| Degeneration of the granular layer | 0.60±0.04$^{A1}$ | 0.33±0.09$^{a2}$ | 0.67±0.08$^{A1}$ | 0.48±0.04$^{b2}$ |
| Necrotized area in the stratum album centrale | 0.70±0.11$^{A1}$ | 0.14±0.08$^{a2}$ | 0.72±0.13$^{A1}$ | 0.69±0.12$^{b1}$ |
| Necrotized area in the stratum griserum centrale | 0.68±0.13$^{A1}$ | 0.13±0.09$^{a2}$ | 0.75±0.12$^{A1}$ | 0.35±0.08$^{b2}$ |
| Necrotized area in the stratum griserum superficiale | 0.47±0.12$^{A1}$ | 0.13±0.03$^{a2}$ | 0.42±0.04$^{A1}$ | 0.35±0.10$^{b1}$ |
| Degeneration in the stratum opticum | 0.33±0.13$^{A1}$ | 0.14±0.04$^{a2}$ | 0.33±0.08$^{A1}$ | 0.18±0.04$^{a2}$ |

Based on an ordinal scale adapted from Bowker et al. (2013): 0 = no detectable alteration; 1 = normal with less than 5% of tissue alterations; 2 = mild with 5–15% of tissue alterations; 3 = moderate with 15–25% of tissue alterations; 4 = marked with 25–50% of tissue alterations and 5 = severe with more than 50% of tissue alterations. The scores represent the mean of six observations (mean ± standard deviation) for each organ of the respective group. The control feed-fed group showed no detectable alterations. 1-2: Values with different numerical superscripts within a row for a particular tissue histopathological change and treatment (dose) differed significantly (P<0.05). A-B, a-b: Values with different alphabetical superscripts for a particular row across treatment groups (1× and 10× doses) on a specific day differed significantly (P<0.05). ED: EB-dosing; PED: Post-EB-dosing. #Glomerulopathy includes a shrunken glomerulus and/or a fragmented glomerulus accompanied by dilation of Bowman's space

**Table 3:** Uptake and depletion of residues of emamectin benzoate (EB) in muscle tissues of *Oreochromis niloticus* fry dosed at 50 µg/kg biomass/day (1×) and 500 µg/kg biomass/day (10×) for 7 days and monitored for 42 post-dosing days

| Treatment period | EB residues in ng/g | |
|---|---|---|
| | 1× group | 10× group |
| Day 0 | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ |
| Day 1 ED | 1.22 ± 0.09$^{b1}$ | 7.13 ± 0.31$^{b2}$ |
| Day 3 ED | 2.51 ± 0.47$^{c1}$ | 9.51 ± 0.36$^{c2}$ |
| Day 7 ED | 4.18 ± 0.40$^{d1}$ | 11.43 ± 0.25$^{d2}$ |
| Day 7 PED | 0.57 ± 0.26$^{a1}$ | 0.87 ± 0.02$^{e1}$ |
| Day 14 PED | 0.13 ± 0.09$^{a1}$ | 0.36 ± 0.35$^{a1}$ |
| Day 21 PED | 0.14 ± 0.09$^{a1}$ | 0.66 ± 0.13$^{a1}$ |
| Day 35 PED | 0.08 ± 0.09$^{a1}$ | 0.48 ± 0.07$^{a1}$ |
| Day 42 PED | 0.12 ± 0.10$^{a1}$ | 0.21 ± 0.02$^{a1}$ |

ED: EB-dosing; PED: Post-EB-dosing; No EB residues were detected in the control group and day 0. Within a column for a given dose, values sharing the same alphabetical superscript (a–e) were not significantly different (P>0.05). Within a row for a specific exposure period, values sharing the same numerical superscript (1–2) did not differ significantly (P>0.05)

### *Uptake and Depletion of EB Residues in Muscle Tissues*

The sample size provided high statistical power ($1 - \beta \geq 0.95$) for EB residue accumulation and depletion across both time-wise and dose-wise comparisons. Muscle tissue EB residues in the 1× and 10× groups showed a significant rise from day 1 onward, reaching 4.18±0.40 ng/g and 11.43±0.25 ng/g, respectively, on day 7. After dosing was suspended, residue levels declined sharply in both groups, although trace amounts remained detectable up to day 42 of PED (Table 3). The residue levels did not differ significantly across PED days in either group (P>0.05).

## Discussion

Our study evaluated how the EB was accrued and cleared in the fry muscle following a seven-day

administration at recommended and ten-fold overdosing doses, alongside their impact on pathophysiological response indicators. Fry administered the 1× dose, exhibited no discernible behavioural alterations, except for a modest decline in feed intake (10.75%). While the 10× group exhibited aberrant swimming, impaired equilibrium, reduced activity, gasping, darkened pigmentation, and a diminished feeding response, with a 28.50% reduction in feed intake, as well as alterations in the internal organs. Earlier investigations also reported similar impaired swimming coordination, lethargy, reduced appetite, and dark pigmentation in temperate fish, *S. salar* (Stone et al., 2002), and *O. mykiss* (Roy et al., 2000), and tropical fish, *Lates calcarifer* fingerlings (Anandaraja et al., 2020), and *O. niloticus* juveniles (Julinta et al., 2020a). Reduction in feeding activity correlated with the dose-dependent survival, consistent with previously reported findings (Julinta et al., 2020a). Conversely, the mortality levels reported by Julinta et al. (2020a) for juveniles exceeded those observed in our study with the fry stage. Also, the mortalities observed during the 2 weeks of the feeding trial were comparatively higher (Singha et al., 2022). The results implied that the EB can be lethal to fry, particularly at a higher dose. Conversely, several previous investigations involving temperate fish species documented an absence of mortality (Bowker et al., 2013; Stone et al., 2002; Roy et al., 2000) or 2-5% mortalities in *S. salar* due to ivermectin (Palmer et al., 1987). Although feed intake and weight gain improved, mortality differences remained significant during the PED period. The recommended EB dosing showed a growth increment of 10.50% compared to the control, and vice versa when overdosed by 9.85%. In contrast, *O. niloticus* juveniles subjected to various EB concentrations over a prolonged 21-day feeding trial showed a significant decline in biomass, fluctuating between 6.91% and 12.18%. Furthermore, the administration of EB at the 1× dose did not produce any statistically significant growth-promoting results (Julinta et al., 2020a-b). The fry remained behaviourally normal and showed no abnormalities, with a noticeable improvement in feeding interest toward the latter part of the PED, aligning with earlier research (Julinta et al., 2020a; Bowker et al., 2013). Previous studies, however, reported mixed outcomes during the PED. For instance, Roy et al. (2000) showed no recovery of EB in *S. salar* at higher doses, whereas improvements in body weight and feed intake were found in *O. niloticus* juveniles (Julinta et al., 2020a-b) and in fries (Singha et al., 2022). In contrast, *O. mykiss* (Roy et al., 2000) showed no significant change in weight gain during the PED.

The Veterinary Drugs Directorate of Health Canada, together with the Canadian Food Inspection Agency, has designated a Maximum Residue Limit (MRL) for EB at 100 ng/g in fish tissues intended for human consumption (Health Canada, 2022). In our study, residue uptake was observed on the very first day in the muscle of fry grown at water temperatures ranging from 26.50 to 27.09 °C, reaching a peak on the last day of ED. Yet, the residue levels were well within the permissible limit set by various international organisations (Health Canada, 2022; JECFA, 2014; ACRDP, 2013; EMEA, 2003) and lower than those observed in *O. niloticus* juveniles (Julinta et al., 2020a). In contrast, Sevatdal et al. (2005) reported higher EB concentration, detected with 74.8 ng/g in the muscle of *S. salar* after one week of ED at colder temperature (7 ± 1°C). These results suggested that the uptake and decline of muscle residues are temperature-dependent. It appears that *O. niloticus* fry has a mechanism to metabolise and eliminate EB from tissues more quickly in tropical conditions. Furthermore, low levels of residues (0.12 ± 0.10 ng/g) were detected in the 1× group on day 42 PED, which indicated prolonged perseverance of EB in the tissues and raised the possibility of subtle effects on growth and other physiological processes. Residue levels of 0.022 ng/g on day 45 PED in *O. niloticus* have been previously reported, supporting the present results (Julinta et al., 2020a). In contrast, substantially higher EB residues have been noted in *S. salar*, with concentrations of 60.5 ng/g on day 1 and 7.3 ng/g on day 45 post-treatment (Whyte et al., 2011). Similarly, residue levels of 21.0 ± 0.0 ng/g on the 7$^{th}$ day of EB-dosing and 2.3 ± 0.0 ng/g on the 28$^{th}$ day of PED were reported in *L. calcarifer* administered the recommended dose (Anandaraja et al., 2020). Even in the 10× group, the concentrations of EB residues did not exceed the safety thresholds (Health Canada, 2022; JECFA, 2014; ACRDP, 2013; EMEA, 2003), indicating a rapid metabolism of EB. Most major aquaculture-regulating regions with comparable restrictions apply higher MRLs of 100 ng/g and mandate shorter depletion phases for temperate fish, such as salmon. For instance, the United Kingdom and Chile have established a 0-day withdrawal interval, while Norway requires 7 days (ACRDP, 2013). The consistent presence of EB residues below the MRL in the fry suggested a zero-day withdrawal period, given their ability to tolerate and metabolise effectively even at an overdose of EB.

The significant, dose-dependent rise in glucose levels during the first week of ED reflects the extent of stress experienced by the fry. The 1.37-2.24-fold increment in glucose suggested that the EB, in both dosing groups, is a probable stressor. The results supported earlier studies on the effects of EB in *O. niloticus* juveniles (Julinta et al., 2020b) and fry (Singha et al., 2022), and abamectin in fish (El-Said, 2007). An increase in glucose levels (hyperglycemia) in the muscle leads to increased energy demand and disturbances in carbohydrate metabolic pathways (Singha et al., 2022; Firat et al., 2011), which might have impacted fry growth in the 10× group. The

glucose levels declined markedly and returned to baseline within a month of PED, while it was still higher in the 10× group even on day 42 of PED, suggesting a persistent effect of EB-induced stress. The marked decline in calcium levels, by roughly 1.13-fold in the 1× group and 1.20-fold in the 10× group, within one week of ED, indicated the onset of hypocalcemia in the fry. This pattern may indicate impaired active transport and calcium regulation, or changes in muscle membrane permeability, similar to effects reported for other pesticides (Atli and Canli, 2007). Conversely, El-Said (2007) found no significant change in plasma calcium levels when exposed to abamectin for one week in *O. niloticus*. On day 42 PED, the calcium in the 1× group increased significantly and was comparable to that of the control group, signifying the recovery of permeability of the muscle for calcium homeostasis. In contrast, the 10× group's calcium was still significantly lower, indicating the inability of the fry to reestablish the active transportation of ions and calcium homeostasis. During the ED phase, muscle chloride levels decreased significantly by roughly 1.12-fold in the 1× group and 1.18-fold in the 10× group. These findings suggested a hypochloremic state in the fry due to EB toxicity, likely resulting from the suppression of active ion-uptake mechanisms (Singha et al., 2022; Firat et al., 2011). It took almost 6 weeks for the chloride levels to reach normalcy in the 1× group. The findings indicated that EB, even at the recommended dose, may interfere with calcium and chloride homeostasis in muscle, thereby potentially impairing growth and productivity. A significant increment in muscle ALP by almost 1.10-fold in the 1× group and 1.87-fold in the 10× group on day 7 of ED was observed. These results in response to EB toxicity suggested hepatic damage, inhibition of bile excretion, and cell necrosis (Abraham et al., 2022; Chimela et al., 2014). The present study's findings are comparable with those of Kushwaha et al. (2020), who reported elevated plasma ALP activity in *O. mossambicus* following 48 hours of abamectin exposure. After six weeks of PED, ALP levels in both groups remained elevated, indicating a prolonged effect of EB on this enzymatic pathway. This persistent rise suggested leakage of hepatic enzymes due to cellular damage (Singha et al., 2022) and a concurrent impairment in the pathways promoting gluconeogenesis from amino acid precursors (Chimela et al., 2014).

The ED caused a significant inhibition of muscle SOD activity, by almost 5-fold at the 1× dose. In the 10× group, no SOD was detected. The decline in antioxidant activity may have compromised the fry's ability to detoxify EB, thereby amplifying its cellular toxicity, as supported by histopathological evidence. The diminished levels of SOD suggested the induction of oxidative stress, driven by increased free radical generation, causing disorders and impaired antioxidant defence mechanisms in the muscle. The pronounced decline in SOD levels may reflect suppression of the antioxidant defense mechanism, although the uniformity of responses limits inferential interpretation. The findings on decrement are consistent with previous reports of SOD inhibition, which have been linked to the sustained accumulation of superoxide radicals and the resulting increase in intracellular hydrogen peroxide ($H_2O_2$) (Abraham et al., 2024; Ajima et al., 2017; Meng et al., 2014) and cell membrane disruption (Karadag et al., 2014). Also, Ogueji et al. (2020) reported significant elevations in SOD activity in fish following exposure to ivermectin. Nevertheless, SOD activity in the 1× group approached near-normal levels by day 42 PED, indicating restoration of cellular membrane integrity and balanced free-radical metabolism. Whereas a noteworthy reduction in activity was noted in the 10× group, implying prolonged disruption of internal homeostasis and sustained $H_2O_2$ accumulation attributable to EB toxicity. AChE activity in the brain tissue decreased by 1.59-fold in the 1× group and by 3.22-fold in the 10× group on day 7 of ED, indicating that dietary EB exerts a quantifiable impact on neural function. As per Kennedy et al. (2014), the reduction in AChE may modulate gamma-aminobutyric acid neurotransmission, potentially compromising growth performance, appetite regulation, and ethological patterns in teleosts. A decline in AChE activity may facilitate acetylcholine accumulation at synapses, potentially triggering neuronal hyperexcitability (Almeida et al., 2005). The outcomes of the present study are consistent with earlier findings that report comparable AChE inhibition patterns in the fish brains upon pesticide exposure (Ghazala et al., 2014) and EB dosing (Abraham et al., 2024; Singha et al., 2022). After dosing was terminated, AChE activity increased in both groups. However, baseline levels were not restored, even in fish fed the recommended dose. This suggested that the effects of EB on neural function may persist longer. The statistical power exceeding 0.8 or 0.9 in the treatment groups for each parameter suggested that the observed differences are robust and not constrained by sample size. However, comparatively lower power observed for certain biochemical variables may reflect tighter physiological regulations under the experimental conditions rather than methodological limitations. Accordingly, these parameters should be interpreted with caution. Further studies with larger sample sizes or extended administration/exposure durations may help refine the understanding of parameters with lower sensitivity (Uttley, 2019).

The liver of fry fed the 1× dose showed meagre glycogen-type vacuolation compared to the overdose group, which is among the most reported toxicological effects (Wolf and Wheeler, 2018). Increased glycogen-type vacuolation, represented by enhanced energy storage

or degenerative changes, could be a toxicological response in fish (Wolf and Wheeler, 2018). The presence of glycogen-type vacuolar degeneration likely reflects the intense metabolic strain on the liver as it works to eliminate EB-related toxins during detoxification. In contrast, only mild to moderate glycogen-type vacuolation and hepatocellular degeneration were reported in *O. niloticus* juveniles fed EB for one week (Abraham et al., 2022; Julinta et al., 2020a). Alike, liver degeneration and vacuolation were noted in the hepatocytes of EB-fed *O. mykiss* fingerlings at 150 µg/kg biomass/day (Bowker et al., 2013). Hepatocyte vacuolization may indicate a disparity between the rate of synthesis of substances within the parenchyma and their subsequent release into the circulatory system. The observation of mild hepatocellular necrosis, characterized by cytoplasmic hypereosinophilia with or without condensation, irregular or rounded cytoplasmic margins, and distinct nuclear alterations, even at the recommended dose, indicated pronounced cellular death in the liver. Such damage can severely compromise hepatic function. A damaged liver cell membrane may lead to the release of cytosolic enzymes into the circulation, which are key markers of toxicity (Abraham et al., 2022; Wolf and Wheeler, 2018). In contrast, neither *S. salar* nor *O. mykiss* exhibited any characteristic lesions associated with EB toxicity during gross necropsy or histopathological evaluation (Stone et al., 2002; Roy et al., 2000). The decline in SOD activity could be linked to EB-induced disturbances in hepatic membrane integrity and cellular homeostasis. The hepatocytes became almost normal, with hepatic cords, normal portal tracts, and central veins, in the livers of fry after 6 weeks of PED at the recommended dose, indicating the reversibility of EB-induced changes after the stress is removed.

In fish, the kidneys contain components of several physiological systems, including tissues with haematopoietic, immunological, endocrine, and urinary functions (Wolf et al., 2015). Though the kidneys of the 1× group fry depicted histopathological alterations, they were trivial. The presence of degenerated renal tubules and necrosis in this group indicated the vulnerability of fry even at the recommended dose regimen. Equally, Julinta et al. (2020a) noted subtle histopathological changes with the disintegration of renal tubules, tubular degeneration with a lack of necrosis, and dilated Bowman's space within a week of dosing at the recommended dose in *O. niloticus* juveniles. The necrosis and vacuolation in the renal tubules may impair ion pump function in tubular cells, suggesting the renal cells were unable to cope with functional disturbances induced by toxicants. Also, mineral deposition was observed in the renal tubules of the 1× group, indicating an imbalance in calcium and chloride ions during the ED. Also, these meagre histological changes in the kidneys of this group may reflect tolerance to the EB diet. Similar changes were observed in the 10× group with a mildly fragmented glomerulus and the necrotised haematopoietic area. In contrast, mild to marked changes with shrunken and fragmented glomeruli and the disintegration of renal tubules with necrotised renal interstitium were documented in *O. niloticus* juveniles at a similar dose (Abraham et al., 2022; Julinta et al., 2020a). Renal damage is expected to disrupt the osmoregulatory function of the fry, and excessive doses may directly affect survival, growth, and physiology (Takvam et al., 2021). The haematopoietic area was observed along with other changes upon cessation of ED, although with necrosis, indicating the retrieval of kidney tissues in both groups. A low EB dose also triggered early tubular regeneration in the kidneys of *O. niloticus* juveniles within one week of ED (Julinta et al., 2020a). Also, regeneration of tubules at the posterior kidneys in *O. mykiss* fingerlings was noted after the suspension of ED (Bowker et al., 2013). On day 42 PED, the regeneration of the tubules, including the recovery of the glomerulus and necrosis in the fry kidneys, was fully achieved. It is suggested that the regeneration process and the re-establishment of internal homeostasis in the kidneys of fry may take several weeks to complete after ED withdrawal, raising a notable concern.

EB administration for 7 days at the 1× dose resulted in subtle histological changes within the intestinal mucosa. The observed epithelial degradation potentially represented a defensive response by the gut to exclude the drug during the ED phase. However, more severe pathologies, specifically mucinous breakdown and the disappearance of absorptive vacuoles, indicated a compromise of the primary mucosal barrier. Also, the unveiling of unicellular goblet cells indicated disruption of their defence mechanism against ED, leading to alterations in physiological functions. These cells are responsible for the production and secretion of high-molecular-weight glycoproteins, such as mucins, which help maintain intestinal homeostasis (Anandaraja et al., 2022). The injuries may affect the absorptive functions of intestinal tissues, which are involved in nutrient transport along the length of the intestine. Conversely, previous research involving *O. niloticus* juveniles administered EB documented intestinal inflammation and epithelial breakdown (Julinta et al., 2020a). However, the extent of tissue changes was great in the 10× group, as reported in our previous studies (Abraham et al., 2022; Julinta et al., 2020a), thus showing dose-dependent EB toxicity. The structural deterioration and cell death observed in the intestinal layers of EB-fed fry could impair oxygen delivery to tissues or lead to elevated acetylcholine levels. Contrarily, research on *O. mykiss* indicated an absence of histological deviations in hepatic, muscular, and intestinal tissues following a 14-day EB feeding regimen (Bowker

et al., 2013). The alterations in the intestinal tissues persisted throughout the 6 weeks of PED at the recommended dose, showing the persistent toxicity of dietary EB. These histoarchitectural changes revealed that EB can alter intestinal tissues and disrupt their physiology, making the intestine a valuable biomarker for toxicological studies.

EB-treated fry exhibited dose-dependent histopathological changes in brain tissue, which correlated with temporal AChE depletion. Within the stratified layers of the optic tectum, these findings support the neurotoxic potential of dietary EB (USDA, 2010) and its capacity to disrupt neuronal function. Further, in the 1× group, neuronal cells appeared dispersed and elongated, while the cells of the 10× group degenerated and shrunken, suggesting that ED can affect the neuronal cells dose-dependently. Probably, the EB residues might have blocked the sodium channel, or else crossed the BBB and entered cells and the nuclear compartments, leading to the dysfunction of the nervous system of fry, similar to that observed with ivermectin in the brain tissue of *Sparus aurata* (Katharios et al., 2004). On day 42 PED, restoration of neuronal cells was evidenced by normalization of nuclear morphology in brain tissue, along with the regeneration of all three central layers, without signs of necrosis. These findings suggested that the fry retain a remarkable ability to replace damaged neurons and initiate neuronal regeneration, even after injuries to the adult central nervous system. Also, with the termination of ED and improvement in brain histoarchitecture, AChE began to recover. Overall, the results suggested that dietary EB at the recommended dose can induce histopathological changes in the kidney, liver, intestine, and brain, and disrupt the physiological responses that might serve as appropriate toxicological biomarkers for EB's dietary or in-feed applications or exposure. Nevertheless, the changes were reversible upon withdrawal of dosing. Findings from the EB dosing studies in healthy fry demonstrated a dose-dependent pattern of renal, hepatic, intestinal, and neurological impairment, aligning with earlier reports (Singha et al., 2022; Julinta et al., 2020a-b).

## Conclusion

The results demonstrated that the uptake and depletion of EB residues in *O. niloticus* at the fry stage following oral administration can affect tolerability, physiological and enzymic responses, and tissue histoarchitecture. Elevated levels of glucose and ALP, and the inhibition of SOD, calcium, chloride and AChE, indicated that EB, even the recommended dose, can significantly disturb the integrity of the cellular membranes of vital organs' functions. Histopathological examinations of EB-fed fry revealed damage in multiple organs. Collectively, these observations underscore the need for caution in applying EB in aquaculture to mitigate potential health risks to fish. The reversibility of measured parameters in the recommended dose group after treatment cessation indicated a degree of resilience in fry under tropical temperature conditions (26.50–27.09ºC). The baseline data generated by this investigation on EB tolerance at 50 µg/kg biomass/day for 7 consecutive days serve as a critical foundation for establishing science-based protocols. These findings may facilitate the development of rigorous advisories for the responsible application of antiparasitic agents in aquaculture, ultimately mitigating potential risks to public health and food safety.

## Acknowledgment

The authors wish to thank the Vice-Chancellor of West Bengal University of Animal and Fishery Sciences, Kolkata, for generously providing the necessary infrastructure and resources to carry out this research.

## Funding Information

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

## Author's Contributions

**Jasmine Singha:** Formal analysis, laboratory investigation, data curation, software, writer original draft.

**Thangapalam Jawahar Abraham:** Conceptualization, methodology, project administration, supervision, resources, funding acquisition, writer review and edited.

**Satyen Kumar Panda:** Formal analysis, data curation, validation, software.

**Prasanna Kumar Patil:** Conceptualization, methodology, project administration, and funding acquisition.

### *Conflict of Interest*

The authors declare no known financial, non-financial, professional, or personal conflicts of interest that could have influenced the work reported in this paper.

### *Disclosure Statement*

No competing interests are declared by the authors.

### *Data Availability*

The data supporting this study are available from the authors on request.

## References

Abraham, T. J., Julinta, R. B., & Patil, P. K. (2022). 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. *Archives of Ecotoxicology, 4*(4), 94–104. https://doi.org/10.36547/ae.2022.4.4.94-104

Abraham, T. J., Singha, J., Bardhan, A., Karmakar, S., Rajisha, R., Panda, S. K., & Patil, P. K. (2024). In-feed antiparasitic veterinary drug emamectin benzoate impairs acetylcholinesterase activity and brain tissue histoarchitecture of *Oreochromis niloticus* fry. *Emerging Animal Species*, *10*, 100042. https://doi.org/10.1016/j.eas.2024.100042

ACRDP. (2013). *Depletion of emamectin benzoate (SLICE®) from skeletal muscle and skin tissues of Atlantic salmon (Salmo salar). Aquaculture, Biotechnology and Aquatic Animal Health Science Branch, Fisheries and Oceans Canada, Ottawa. Aquaculture Collaborative Research and Development Program Fact Sheet 21*.

Ajima, M. N. O., Pandey, P. K., Kumar, K., & Poojary, N. (2017). Neurotoxic effects, molecular responses and oxidative stress biomarkers in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), exposed to verapamil. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *196*, 44–52. https://doi.org/10.1016/j.cbpc.2017.03.009

Almeida, L. C. de, Aguiar, L. H., & Moraes, G. (2005). Effect of methyl parathion on the muscle and brain acetylcholinesterase activity of matrinxã (*Brycon cephalus*). *Ciência Rural*, *35*(6), 1412–1416. https://doi.org/10.1590/s0103-84782005000600029

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

Anandaraja, R., Patil, P. K., Avunje, S., Kumaran, M., Solanki, H. G., Jithendran, 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

Atli, G., & Canli, M. (2007). Enzymatic responses to metal exposures in a freshwater fish, *Oreochromis niloticus*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *145*(2), 282–287. https://doi.org/10.1016/j.cbpc.2006.12.012

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

Chimela, W., Mesua, N., & Abdulraheem, B.-A. (2014). Alkaline phosphatase (ALP) activity in selected tissues and organs of *Clarias gariepinus* exposed to different levels of paraquat. *Journal of Environmental and Analytical Toxicology*, *04*(03), 1000214. https://doi.org/10.4172/2161-0525.1000214

European Commission. (2002). Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities*, *L 221*(11–12), 8–36

European Commission. (2021). Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the Performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results, as well as on the methods to be used for sampling and repealing decisions 2002/657/EC and 98/179/EC. *Official Journal of the European Communities*, *L 180*, 84-109

CPCSEA. (2021). *Guidelines of CPCSEA for experimentation on fishes. Committee for the Purpose of Control and Supervision of Experiments on Animals. Animal Welfare Division, Ministry of Environment, Forest and Climate Change, Government of India*.

El-Said, M. M. (2007). Evaluation of abamectin toxicity on some biochemical constituents and osmoregulation in freshwater fish *Oreochromis niloticus (Tilapia niloticus). Journal of the Egyptian Society of Clinical Toxicology*, *37*, 1–10.

EMA. (2018). *Guideline on determination of withdrawal periods for edible tissues. EMA/CVMP/SWP/735325/2012, Committee for Medicinal Products for Veterinary Use (CVMP), European Medicines Agency, European Union Agencies Network, Amsterdam, The Netherlands*.

EMEA. (2003). *Committee for Veterinary Medicinal Products. Summary report: Emamectin (1). European Medicines Agency. Report no. EMEA/MRL/546/99-FINAL*.

FAO. (2024). *The State of World Fisheries and Aquaculture 2024 – Blue Transformation in action*. https://doi.org/https://doi.org/10.4060/cd0683en

Fırat, Ö., Cogun, H. Y., Yüzereroğlu, T. A., Gök, G., Fırat, Ö., Kargin, F., & Kötemen, Y. (2011). A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*. *Fish Physiology and Biochemistry*, *37*(3), 657–666. https://doi.org/10.1007/s10695-011-9466-3

Ghazala, Mahboob, S., Ahmad, L., Sultana, S., AlGhanim, K., Al-Misned, F., & Ahmad, Z. (2014). Fish cholinesterase as biomarkers of sublethal effects of organophosphorus and carbamates in tissues of *Labeo rohita. Journal of Biochemical and Molecular Toxicology*, *28*(3), 137–142. https://doi.org/10.1002/jbt.21545

Health Canada. (2022). List of maximum residue limits (MRLs) for veterinary drugs in foods. *Departments and Agencies*. https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/maximum-residue-limits-mrls/list-maximum-residue-limits-mrls-veterinary-drugs-foods.html

JECFA. (2014). *Toxicological evaluation of certain veterinary drug residues in food. Seventy-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO, Geneva. 241p.*

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

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 Nile tilapia *Oreochromis niloticus* (L.) juveniles. *Journal of Environmental Biology*, *41*, 973–979. http://doi.org/10.22438/jeb/41/5/MRN-1222

Karadag, H., Fırat, Ö., & Fırat, Ö. (2014). Use of Oxidative Stress Biomarkers in Cyprinus carpio L. for the Evaluation of Water Pollution in Ataturk Dam Lake (Adiyaman, Turkey). *Bulletin of Environmental Contamination and Toxicology*, *92*(3), 289–293. https://doi.org/10.1007/s00128-013-1187-0

Katharios, P., Pavlidis, M., & Iliopoulou-Georgudaki, J. (2004). Accumulation of ivermectin in the brain of sea bream, Sparus aurata after intraperitoneal administration. *Environmental Toxicology and Pharmacology*, *17*(1), 9–12. https://doi.org/10.1016/j.etap.2004.01.003

Kennedy, C. J., Tierney, K. B., & Mittelstadt, M. (2014). Inhibition of P-glycoprotein in the blood–brain barrier alters avermectin neurotoxicity and swimming performance in rainbow trout. *Aquatic Toxicology*, *146*, 176–185. https://doi.org/10.1016/j.aquatox.2013.10.035

Kumar, V., Swain, H. S., Das, B. K., Roy, S., Upadhyay, A., Ramteke, M. H., Kole, R. K., & Banerjee, H. (2022). Assessment of the effect of sub-lethal acute toxicity of Emamectin benzoate in *Labeo rohita* using multiple biomarker approach. *Toxicology Reports*, *9*, 102–110. https://doi.org/10.1016/j.toxrep.2022.01.001

Kushwaha, S., Anerao, I., Rajput, S., Bhagriya, P., & Roy, H. (2020). Evaluation of abamectin-induced hepatotoxicity in Oreochromis mossambicus. *Cogent Biology*, *6*(1), 1761277. https://doi.org/10.1080/23312025.2020.1761277

Limbu, S. M., Chen, L., Zhang, M., & Du, Z. (2021). A global analysis on the systemic effects of antibiotics in cultured fish and their potential human health risk: a review. *Reviews in Aquaculture*, *13*(2), 1015–1059. https://doi.org/10.1111/raq.12511

Meng, S. L., Chen, J. Z., Xu, P., Qu, J. H., Fan, L. M., Song, C., & Qiu, L. P. (2014). Hepatic antioxidant enzymes SOD and CAT of Nile tilapia (*Oreochromis niloticus*) in response to pesticide methomyl and recovery pattern. *Bulletin of Environmental Contamination and Toxicology*, *92*(4), 388–392. https://doi.org/10.1007/s00128-014-1232-7

Ogueji, E., Nwani, C., Mbah, C., Iheanacho, S., & Nweke, F. (2020). Oxidative stress, biochemical, lipid peroxidation, and antioxidant responses in *Clarias gariepinus* exposed to acute concentrations of ivermectin. *Environmental Science and Pollution Research*, *27*(14), 16806–16815. https://doi.org/10.1007/s11356-019-07035-4

Padros, F., Gourzioti, E., Fabris, A., Figenschou, A., Manfrin, A., Metselaar, M., Palić, D., & Vesanto, S. (2024). Relevance of pharmacovigilance in aquatic animal medicine. A perspective from aquaculture health experts attending a focus group meeting at the EMA. *Bulletin of the European Association of Fish Pathologists*, *44*(2), 122939. https://doi.org/10.48045/001c.122939

Palmer, R., Rodger, H., Drinan, E., Dwyer, C., & Smith, P. R. (1987). Preliminary trials on the efficacy of ivermectin against parasitic copepods of Atlantic salmon. *Bulletin of the European Association of Fish Pathologists*, *7*(2), 47–54.

Patil, P. K., Geetha, R., Mishra, S. S., Abraham, T. J., Solanki, H. G., Krupesha Sharma, S. R., Pradhan, P. K., Manna, S. K., Avunje, S., Abhinaya, D., Felix, K. T., Vinay, T. N., Paniprasad, K., Paria, A., Raja, S. A., Saraswathy, R., Sahoo, S. N., Rathod, R., Rameshkumar, P., … Jena, J. K. (2025). Unveiling the economic burden of diseases in aquatic animal food production in India. *Frontiers in Sustainable Food Systems*, *9*, 1480094. https://doi.org/10.3389/fsufs.2025.1480094

Patil, P. K., Mishra, S. S., Pradhan, P. K., Manna, S. K., Abraham, J. T., Solanki, H. G., Shahi, N., Swain, P., Sahoo, S. N., Avunje, S., Sharma, K. S. R., Geetha, R., Priyadharshini, R., Nagaraju, V. T., Paniprasad, K., Kumar, A., Debnath, D., Panikkar, P., Raja, R. A., … Jena, J. (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

Rico, A., Phu, T. M., Satapornvanit, K., Min, J., Shahabuddin, A. M., Henriksson, P. J. G., Murray, F. J., Little, D. C., Dalsgaard, A., & Van den Brink, P. J. (2013). Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture*, *412–413*, 231–243. https://doi.org/10.1016/j.aquaculture.2013.07.028

Rico, A., Vighi, M., Van den Brink, P. J., ter Horst, M., Macken, A., Lillicrap, A., Falconer, L., & Telfer, T. C. (2019). Use of models for the environmental risk assessment of veterinary medicines in European aquaculture: current situation and future perspectives. *Reviews in Aquaculture*, *11*(4), 969–988. https://doi.org/10.1111/raq.12274

Roberts, R. J. (2012). *Fish pathology*.

Roy, W. J., Sutherland, I. H., Rodger, 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*(1–2), 19–29. https://doi.org/10.1016/s0044-8486(99)00307-5

SANCO. (2012). *Method validation and quality control procedures for pesticide residues analysis in food and feed. Document N° SANCO/12495/2011*.

Sevatdal, S., Magnusson, A., Ingebrigtsen, K., Haldorsen, R., & Einar Horsberg, T. (2005). Distribution of emamectin benzoate in Atlantic salmon (*Salmo salar* L.). *Journal of Veterinary Pharmacology and Therapeutics*, *28*(1), 101–107. https://doi.org/10.1111/j.1365-2885.2004.00629.x

Singha, J., & Abraham, T. J. (2025). Dietary emamectin benzoate alters the gill and spleen histoarchitecture of monosex *Oreochromis niloticus* fry. *Journal of Fisheries*, *13*(2), 132401. https://doi.org/10.17017/j.fish.1001

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 & Pharmacology*, *252*, 109223. https://doi.org/10.1016/j.cbpc.2021.109223

St-Hilaire, S., Cheng, T. H., Chan, S. C. H., Leung, C. F., Chan, K. M., Lim, K. Z., Furtado, W., & Bastos Gomes, G. (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

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* (Krøyer). *Aquaculture*, *210*(1–4), 21–34. https://doi.org/10.1016/s0044-8486(01)00822-5

Singha, J., Abraham, T. J., Roy, A., Dash, G., Nagesh, T. S., & Patil, P. K. (2021). Use of aquadrugs, chemicals and biological products in freshwater aquaculture systems of West Bengal, India. *Journal of Fisheries and Life Sciences*, *6*(1), 7–18.

Takvam, M., Wood, C. M., Kryvi, H., & Nilsen, T. O. (2021). Ion transporters and osmoregulation in the kidney of teleost fishes as a function of salinity. *Frontiers in Physiology*, *12*, 664588. https://doi.org/10.3389/fphys.2021.664588

USDA. (2010). *Emamectin benzoate human health and ecological risk assessment final report (October 28, 2010). United States Department of Agriculture.*

USFDA. (2014). The antiparasitic resistance management strategy group. American Veterinary Medical Association: United States Food and Drug Administration.

Uttley, J. (2019). Power analysis, sample size, and assessment of statistical assumptions-Improving the evidential value of lighting research. *LEUKOS*, *15*(2–3), 143–162. https://doi.org/10.1080/15502724.2018.1533851

Whyte, S. K., Westcott, J. D., Byrne, P., & Hammell, K. L. (2011). Comparison of the depletion of emamectin benzoate (SLICE®) residues from skeletal muscle and skin of Atlantic salmon (*Salmo salar*), for multiple dietary dose regimens at 10°C. *Aquaculture*, *315*(3–4), 228–235. https://doi.org/10.1016/j.aquaculture.2011.02.043

Wolf, J. C., & Wheeler, J. R. (2018). A critical review of histopathological findings associated with endocrine and non-endocrine hepatic toxicity in fish models. *Aquatic Toxicology*, *197*, 60–78. https://doi.org/10.1016/j.aquatox.2018.01.013

Wolf, J. C., Baumgartner, W. A., Blazer, V. S., Camus, A. C., Engelhardt, J. A., Fournie, J. W., Frasca, S., Groman, D. B., Kent, M. L., Khoo, L. H., Law, J. M., Lombardini, E. D., Ruehl-Fehlert, C., Segner, H. E., Smith, S. A., Spitsbergen, J. M., Weber, K., & Wolfe, M. J. (2015). Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies. *Toxicologic Pathology*, *43*(3), 297–325. https://doi.org/10.1177/0192623314540229

Zhang, T., Dong, Z., Liu, F., Pan, E., He, N., Ma, F., Wang, G., Wang, Y., & Dong, J. (2022). Avermectin induces carp neurotoxicity by mediating blood-brain barrier dysfunction, oxidative stress, inflammation, and apoptosis through PI3K/Akt and NF-κB pathways. *Ecotoxicology and Environmental Safety*, *243*, 113961. https://doi.org/10.1016/j.ecoenv.2022.113961