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Pharmacokinetics of emamectin benzoate and its effect on metabolic and antioxidative enzymes activity in *Labeo rohita*

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Abstract
A pharmacokinetics study of emamectin benzoate (EMB) was conducted for the first time in Indian major carp, *Labeo rohita*, and the physio-biochemical changes to the drug were studied. A single dose of EMB at 20 mg kg^{-1} had administered through feed to *L. rohita* juveniles (100±10 g) and blood samples were collected at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96 and 120 hours after administration to determine the presence of EMB residue in plasma using C-18 reversed phase High-Performance Liquid Chromatography (HPLC). Maximum concentration (C_{max}) of 21.82±0.012 µg ml^{-1} had detected at one hour (T_{max}). The depletion profile of emamectin benzoate indicated that the drug was eliminated in 96 h. The metabolic enzymes (ALT, AST, and LDH) and antioxidant enzymes (SOD, CAT, GST, GPx, and ALP) were significantly increased ($p<0.05$) in all EMB treatments groups compared to control group. All the above biomarkers were peaked on the 6th day and gradually reduced up to the level of control by 15th day, indicating that the physiological disturbance due to the drug was normalized within the above period. Also, the EROD assay, estimation of CYTP4501A induction was also proved to be a sensitive biomarker for indicating the pharmacodynamics response due to the drug, as the activity also showed the same trend as that of the enzymes.

Keywords: Emamectin benzoate, pharmacokinetics, HPLC, *Labeo rohita*

1. Introduction
The random outbreak of disease is a significant constraint in aquaculture resulting in fish mortality and reduced yield (Kumar *et al.*, 2015) [28]. Intensive rearing of fish often creates a highly stressful environment, leading to suppression of immune response and rendering the fish highly susceptible to different diseases (Kumari and Sahoo, 2006) [29]. The occurrence of parasites is frequent in an aquatic organism (Moyle *et al.*, 2006) [35]. Substantial economic losses in parasites infected farms are the result of reduced growth rate and increased mortality (Kayis *et al.*, 2009) [24]. The highest prevalence of parasite infection (94.54%) in *L. rohita* was found to be during winter (Monir *et al.*, 2015) [33]. The intensity of parasitic infection in fish is greatly influenced by seasonality due to altered host physiology (Pennycuick *et al.*, 1972; Rahman *et al.*, 2005) [37, 39]. Intense parasitic infection can cause skin ulceration and difficulties during the ordinary course of reproduction (Rahman *et al.*, 2005) [39].
Emamectin benzoate (4″-deoxy-4″ methylamino derivative of abamectin) has been widely used to control lepidopterous pests in agricultural products (Yen and Lin, 2004) [57]. Emamectin has also been successfully utilised by fish farmers to control the sea lice in *Salmo salar* (Ikeda and Omura, 1997; Rodriguez *et al.*, 2007) [21, 41]. Emamectin has shown efficacy against all life- stages of parasites such as *Lepeophtheirus salmonis* (*Salmon louse*) and *Caligus elongatus*, (Rodriguez *et al.*, 2007) [41].
Pharmacokinetics is one of the major branches of pharmacology and an essential tool to study the interaction of the drug with the body like their distribution, absorption, biotransformation, and excretion (Dar *et al.*, 2018) [7] which also helps to find out the optimum dose of the drug for an animal. Further, the adverse impact on body physiology and metabolism can estimate by evaluating markers of hepatic detoxification. The physio-metabolic responses of juvenile *L. rohita* were evaluated in EMB fed groups. The present pharmacokinetic study was conducted to understand the physio-metabolic responses of EMB in *L. rohita* to fulfil the knowledge gap in the pharmacokinetics of a drug for efficient drug delivery against ectoparasitic infection in *L. rohita* (IMC's).

2. Materials and methods

2.1. Preparation of experimental diets

Semi-purified ingredients including casein, gelatin, dextrin, starch soluble, cellulose, cod liver oil and sunflower oil, carboxymethyl cellulose and butylated hydroxytoluene (BHT) were procured from HiMedia, India and Vitamin mineral mix by Agrimin forte was used to prepare a feed. The experimental feed contains 32.09% crude protein and 6% crude lipid to fulfil the protein and energy need for the fishes and 0.02% emamectin benzoate (EMB, Sigma Aldrich, USA) coated using UNIGEL-500. The control diet was devoid of the EMB but coated in UNIGEL-500.

2.2. Experimental set up and sampling

Healthy juveniles of *Labeo rohita* weighing 100 ± 10 g were randomly distributed in four experimental groups in triplicates, with a stocking density of 15 fishes per tank. The experimental set up consisted of two treatments including EMB receiving group (at 20 mg kg^{-1}) and a control. Acclimated fishes were kept under fasting for a day before starting the experiment. The water temperature of 28 ± 2°C and an oxygen level of 6–8 ppm were maintained throughout the experiment. Fishes were anaesthetised using clove oil, and blood samples were collected aseptically from the anaesthetised animals at intervals of 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, and 120 hours post administration of the drug by piercing caudal vein using a 1 ml syringe. Plasma protein was precipitated by adding I ml of methanol to 200 µl plasma and the supernatant was collected by centrifuging the samples at 3000×g for 10 min. The samples were stored for HPLC analysis after filtering through 0.22 µm nylone syringe filter (Dar *et al.,* 2018) [7]. Liver, muscle and gill tissues samples were collected from treated fishes after 0, 3, 6, 9, 12 and 15 days, homogenised in sucrose solution and centrifuged at 5000 rpm for 10 min at 4 °C and supernatant was stored at -20 °C.

2.3. Quantification of emamectin benzoate from samples

Concentration of EMB in plasma as well as muscle tissue samples was carried out as describe by Roy *et al.* (2006) [43] with slight modification. Twenty microliter aliquots of the filtered fractions of samples were injected into the HPLC system by the Autosampler (Water 2707, USA). Isocratic elution was carried out at a flow rate of 1 ml min^{-1}, using a mobile phase containing 0.1% orthophosphoric acid: acetonitrile (60:40) through a C-18 reversed-phase analytical column (250 mm × 4.6 mm, 5.0 µm) using High-Performance liquid Chromatography (HPLC, Water, USA). Elution of was done at 22 °C and measured at 244 nm using UV-Vis detector. The data were analysed by Water Breeze™ two integrator system.

A calibration curve was plotted with thirteen working standards of EMB with concentrations ranging from 0.05 µg ml^{-1} to 100 µg ml^{-1} and the linearity of the detector response were evaluated (R^2 = 0.97, Fig. 1).

2.4. Metabolic enzymes

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed in plasma by using ERBA lab kit. Lactate dehydrogenase is a key metabolic step in glycolysis and other metabolic pathways (Everse and Kaplan, 1975) [10]. The reaction mixture for LDH contained 2.7 ml of 0.1 M phosphate buffers (pH 7.5), 0.1ml of 2% NADH and 0.1ml of 0.02M sodium pyruvate. The rate of reduction in the optical density of NADH to NAD at 340 nm by liver tissue homogenate was measured.

Alkaline phosphatase (ALP) activity was measured from the serum samples by following Garen and Levinthal (1960) [13] method. Briefly, the assay mixture comprised of 0.2 ml of 0.2 M bicarbonate buffer, 0.1ml of 0.1 M $MgCl_2$, 0.1ml serum sample, 0.5 ml distilled water and 0.1 ml of 0.1 M para-nitrophenyl phosphate. The resultant reaction mixture was incubated at 37 °C for 15 min, and the 1 ml of NaOH stopped the reaction prior reading at 410 nm.

Superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione peroxidase (GPx) enzyme activities were measured to determine the oxidative stress status of fish. The SOD was assayed based on the oxidation of epinephrine-adrenochrome transition. The decrease in the absorbance of the reaction mixture containing bicarbonate buffer, enzyme extract and hydrogen peroxide was measured at 240 nm for 3 minutes to determine catalase activity (Takahara *et al.*, 1960) [52].

Glutathione S-transferase (GST) activity was determined by Habig *et al.,* (1974) [17] method. Briefly, 2.4 ml of 0.3M potassium phosphate buffer (pH 6.9) and 0.1 ml of 30 mM glutathione reduced were added to a cuvette directly. The reaction was initiated by adding glutathione to the mixture and change in absorbance was measured at 340 nm against the bank. Glutathione peroxidase (GPx, Flohe and Gunzler, 1984) [12] was determined by adding 0.2 ml phosphate buffer (pH 7.0, 0.4 M), 0.2 ml EDTA (0.4 mM), 0.2 ml sodium azide (10 mM), 0.1ml glutathione reduced, (0.02M), 0.2 ml glutathione reductase,0.1 ml NADPH, 0.5 ml 10% TCA, 2 ml Tris buffer (pH 8.9, 0.4 M) and 50 µl DTNB. Oxidised glutathione (GSSG), produced upon reduction of an organic hydroperoxide by GPx and is recycled to its reduced state by glutathione reductase and NADPH. Oxidation of NADPH to $NADP^+$ results decreased in absorbance of the resultant mixture at 340 nm, and the rate of decrease is directly proportional to the GPx activity of the sample.

All the assays were expressed in mg of protein in the sample. Aliquots of the sample were taken in dry test tubes, and volume raised to 1 ml by adding distilled water, 250 µl of NaOH and 5 ml of Bradford reagent. The absorbance was taken at 595 nm to find the total protein content in a tissue sample (Bradford, 1976) [1].

2.5. EROD activity assay

The liver tissue was homogenised in four volumes of homogenization buffer containing 0.08 M Na_2HPO_4; 0.02 M KH_2PO_4 and 0.15 M KCl (pH 7.4) followed by centrifuging for 20 minutes at 4 °C with speed of 10,000×g. Ultracentrifugation was carried out using BECKMAN ultracentrifuge (Coulter, U.S.A) at 100,000 ×g for 60 min at 4 °C using the supernatant of initial centrifugation step (Klemz *et al.,* 2010) [25]. One mL of resuspension buffer (the homogenization buffer with 20% glycerol, v/v) was used to resuspend the pellet obtained to obtain the hepatic microsomal fraction. Bradford (1976) [1] method was used to determine the total protein content of the samples. The EROD activity was measured at excitation wavelength of 535 nm and the emission wavelength of 585 nm using spectrofluorometer (RF-5301 PC, Shimadzu). A reaction mixture containing ten µl microsomal protein and 0.097 mg/mL 7-ethoxyresorufin was received 0.1 M NADPH. The resorufin production was measured for 2–3 min by recording the change in fluorescence from the mixture (Nilsen *et al.,* 1998) [36]. The

internal standard used was 10 µl resorufin solution.
EROD activity (picomole of resorufin produced per minute mg protein) = Fs /min × R ×FR × 1 Vs × 1 Cs
Fs/min = Increase in sample fluorescence per min.
R = Amount of resorufin added as an internal standard (pmol).
FR = Increase in fluorescence owing to resorufin standard.
Vs =Sample volume (mL).
Cs = Protein concentration of sample (mg mL^{-1}).

2.6. Statistical analysis
The data were statistically analysed using the statistical package SPSS version 22.0 and were subjected to one-way ANOVA following Duncan's multiple range tests to determine the significant differences between the means.

3. Results
3.1. Depletion profile of EMB
The plasma concentration of EMB after oral administration to *L. rohita* was determined at different time intervals. Figure 3A to 3K is chromatogram of EMB in plasma at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72 and 96hours of post injection and figure 5 A to 5C depicts the chromatogram of EMB of muscle tiissue extract at 3, 6, and 9 days after post injection. Maximum concentration (C_{max}) of EMB (21.82±0.012 µg ml^{-1}) was found at one hour (T_{max}) in, and no EMB detected after 96 h (Fig 2). At the same time no residue existed in muscle tissue after 12 days of feeding the medicated feed containing EMB to *L. rohita (*Fig 4).

3.2. Activity of metabolic enzymes
The activity of transaminase enzymes (AST and ALT) are presented in Fig. 6 and 7. Plasma ALT and AST was higher on the 6th day (22.13± 0.37 IU^{-l} for AST and 27.00± 0 for IU^{-l} ALT). Liver and muscle LDH activity were highest on the 6th day (17.24 ± 0.61 IU^{-l} for muscle and 21.45 ± 0.76 IU^{-l} for liver). Alkaline phosphatase activity in the present study was highest on the 6th day in plasma (14.54 ± 1.19 IU^{-l}).

3.3. Activity of antioxidant enzymes
Highest SOD activity was on the 6th day in both liver (29.21 ± 0.71 IU^{-l}) and gills (24.39 ± 0.15 IU^{-l}). Liver and gills catalase activity was highest on the 6th day (23.51 ± 1.71 IU^{-l} for liver and 20.99± 0.32 IU^{-l} for gills). The highest GST activity was observed on the 6th day in the liver (21.86 ± 0.29 IU^{-l}). The GPx activity was highest on the 6th day in the liver (3.54 ± 0.02 IU^{-l}). All the enzymes reached at a normal level on the 15th day (Fig. 8 to 16).

3.4. EROD activity assay
The EROD activity of the liver microsome after feeding with EMB contained feed at different time intervals in *L. rohita* is shown in Table 1. The EROD activity was highest on the 6th day (25.44 pmol $min^{-1}mg^{-1}$). Bioaccumulation of EMB in fish muscle was highest on the 6th day (0.83±0 µg g^{-1}) and reached at a reasonable level on the 15th day. The activity of EROD is present in Fig. 17.

4. Discussion
There are no response studies of EMB on pharmacokinetic and physio-metabolic activity in tropical fishes. Thus, the present study has undertaken pharmacokinetics and physio-metabolic responses in *L. rohita* for the first time. The pharmacokinetics data of emamectin benzoate is rarely available, and hence the parameters of the present study are compared with the pharmacokinetics data of other drugs to find out the effective dose of emamectin benzoate. This study has provided substantial proof to get an effective dose for *Labeo rohita* which is an essential factor for the preparation of medicated feed.
Quantification of EMB in the HPLC system was carried out with concentration ranging from 0.05 ml^{-1} to100 µg ml^{-1}. A satisfactory linearity correlation coefficient of a calibration curve to be 0.972 was found which was similar as reported by Cox *et al.* (2009) [6] for metronidazole. The significant fact in which HPLC method lies is that HPLC makes the technique simple by performing a direct analysis (Iosifidou *et al*., 1996; Gokbulut *et al.*, 2007; Dar *et al.*, 2017b) [22, 16, 8].
The pharmacokinetic and efficacy studies of emamectin benzoate and its related compounds were carried out in *Gadus morhua* (Samuelsen, 2010; Hamre *et al.*, 2011) [45, 19], *Salmo salar* (Saksida *et al.*, 2010; Lees *et al.*, 2008; Roy *et al.*, 2000; Skilbrei *et al.*, 2008; Stone *et al.*, 2002; Glover *et al.*, 2010) [44, 31, 42, 49, 51, 15] *Dicentrarchus labrax* (Toksen *et al.*, 2006) [53], *Anguilla rostrata* (Larrat *et al.*, 2012) [30], *Oncorhynchus mykiss* (Hakalahti *et al.*, 2004; Roy *et al.*, 2006) [18, 43] for treating parasitic infection. The EMB attained the maximum concentration (C_{max}) of 21.82 ± 0.01µg ml^{-1} in the plasma at 1 h (T_{max}), and then the concentration of EMB gradually dropped and not detected after 96 h. These findings support the earlier studies carried out in *Gadus morhua* by Samuelsen (2010) [45], where C_{max} of EMB was found at 89 h. The total concentration of EMB in plasma in fishes supplemented with medicated feed at 20 mg kg^{-1} of body weight was 18.17% respectively. While adsorption other avermectin derivatives in the gastrointestinal tract of human were only 5-10% (Reynolds, 1996) [40]. Higher absorption may be because of the agastric alimentary canal in rohu, and this indicates the effective absorption of EMB from the gastrointestinal tract of *L. rohita* compare to human.
The maximum plasma concentration of EMB raised to 21.82 ± 0.01 µg ml^{-1} after 20 mg kg^{-1} single dose administration. Similarly, a maximum concentration of 14 ng ml^{-1} in *Salmo salar* (Sevatdal *et al.*, 2005) [47] and 15ng ml^{-1} in *Gadus morhua* (Samuelsen, 2010) [45] were reported when exposed to EMB. While when avermectin expsed to *Ctenopharyngeodon idella* (Qin *et al.*, 2012) [38] and lactating sheep (Cerkvenik *et al.*, 2002) [3], the maximum concentration were found to be 18.51 and 21.7 µg ml^{-1}. Similarly, 0.13 µg ml^{-1} in sheep (Short *et al.*, 1988) and 1.32 ± 0.05 µg ml^{-1} in *L. rohita* (Dar *et al.*, 2018) were reported to be the maximum concentration in plasma when ivermectin exposed to the animal. Maximum concentration of albendazole was 3.46± 0.06 µg ml^{-1} in *Pangasianodon hypophthalmus, when* exposed to 20 mg kg^{-1} of albendazole (Dar *et al.*, 2017b) [8].
While comparing the results of the present study with other animals, it is evident that *L. rohita* can absorb EMB efficiently through the gastrointestinal tract and hence detected in plasma. The plasma concentration of EMB in the present study was found up to 96 h whereas in the earlier study conducted by Samuelsen (2010) [45], who found the plasma concentration of EMB up to 385 h after treatment, and this may be because the absorption rate is higher in monogastric animals (Dar *et al.*, 2018) [7].
AST and ALT are involved in functioning test liver for hepatic injury evaluation (Willianson *et al.*, 1996) [56]. In the present study, the activity of AST and ALT was higher on the 6th days, which means gluconeogenesis provision through

alanine and aspartate for production of glucose which may encompass to reduce the stress of drug-induced (Dar *et al.*, 2018) [7]. Knox and Greengard (1965) [26] reveal that the during stress higher levels of transaminase leads to increased ketoacid production in TCA cycle which affect oxidative metabolism. It is evident from normal level of ALT and AST, that administration of EMB did not cause any metabolic stress in *L. rohita.*

Oxidative stress status of the EMB treated *L. rohita* were determined by the antioxidative enzymes *viz.*, CAT, GST, SOD and GPx. Higher activities of GST, SOD, catalase and GPx found after EMB administration might be due to detoxification mechanism. Similarly, higher ROS production was reported by Kohen and Nyska (2002) [27] with a parallel increase in SOD and primary cellular enzymatic defence against H_2O_2 produced during oxidative stress by increasing CAT activity was reported by Dorval *et al.*, (2003) [9]. The GST detoxifies potential harmful substances, including reactive oxygen species, lipid peroxidation products and electrophilic compounds (Torres-Rivera and Landa, 2008) [54]. The selenium-containing an oxidative enzyme, GPx catalyses the reduction reaction of hydrogen peroxide or lipid peroxidase by reduced glutathione (Cohen and Hochstein, 1963; Christopherson, 1969) [5, 4].

At the same time, a higher level of LDH indicates production of more lactate, which is used during anaerobic metabolism in fish as a source of gluconeogenesis (Moon and Foster, 1995) [34]. The ALP is the indicator of nutrient absorption intensity in enterocytes of fish (Harpaz and Uni, 1999; Gawlicka *et al.*, 2000) [20, 14]. The ALP also plays an essential role in phosphorus metabolism in the body, so an elevated level of ALP in plasma of *Ictalurus punctatus* indicated a higher level of phosphorus in the diet (Eya and Lovell, 1997) [11].

In fish EROD used as a biomarker for cytochrome P4501A1 induction (Whyte *et al.*, 2000) [55]. It is also the best marker for xenobiotic enzymes for the detoxification of drug (Stegeman and Hahn, 1994) [50]. Elevated levels of EROD activity reveals the toxic effect of the body of the animals if the fish presence of heavy metals showed the higher level EROD activity (Jewett *et al.*, 2002; Caselles *et al.*, 2006) [23, 2]. Higher concentration of EMB in muscle was found on the 6th day (0.83±0 µg g^{-1}), and it was not detected on the 15th day. Thus, it is clear that the fish do not contain any residues in the flesh after 15 days of EMB treatment.

Ethics statement

The guidelines of the CPCSEA (Committee for Control and Supervision of Experiments on Animals) was followed the for the treatment with the animals used in this study.

Conflict of Interest

The authors don't have conflict of interest

Consent to publish

The authors are giving consent to the submission to the journal.

Consent to participate: The authors are giving their consent to participate in the publication of manuscript in this journal

Authors' contributions

Diganta Chetia- experimental work and manuscript writing, Showkat Ahmad Dar- manuscript editing and writing, Subodh Gupta- research concept, Tincy Varghese- experimental analysis, Pallath Muhammed Nuzaiba-experimental analysis, Prem.P. Srivastava-manuscript summarization, Parimal Sardar- editing and analysis

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Table 1: Composition of emamectin benzoate based diet.

Ingredients	Control feed (g/100 g)	Medicated feed (g/100g)
Casein	22.11	22.11
Gelatin	14	14
Dextrin	24	24
Starch soluble	22	21.8
Emamectin benzoate	0	0.20
Cellulose	6	6
Cod liver oil	3	3
Sunflower oil	3	3
Vit-min premix	1.50	1.50
Vitamin C	0.38	0.38
CMC	3	3
BHT	0.01	0.01
Choline Chloride	0.5	0.5
Betaine	0.5	0.5
Total	100	100

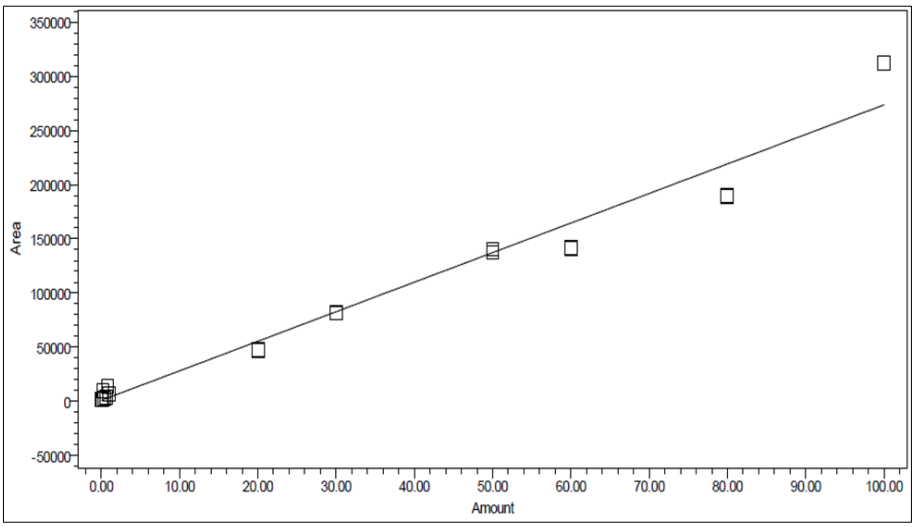


Fig 1: The calibration curve of EMB standards solution of various concentration range from 0.05 to 100 µg ml^{-1}.

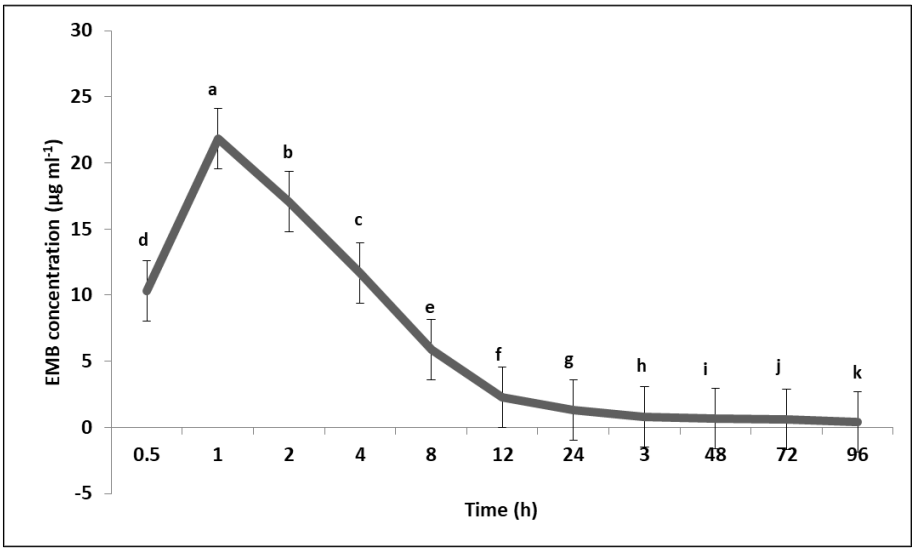
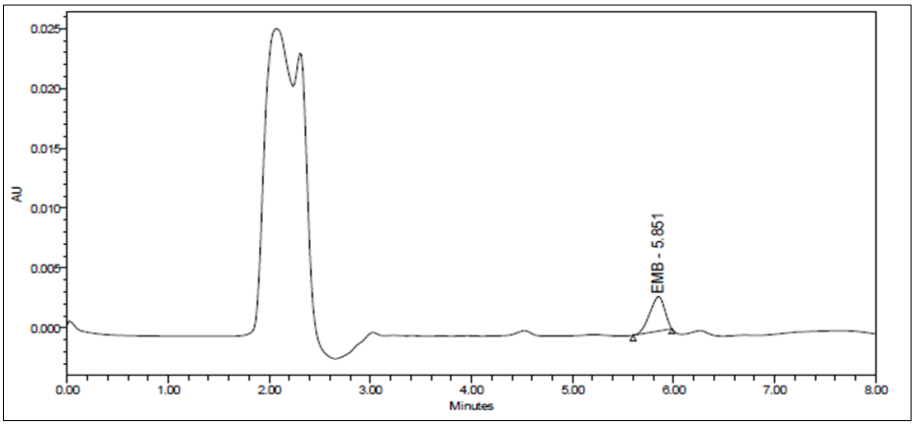
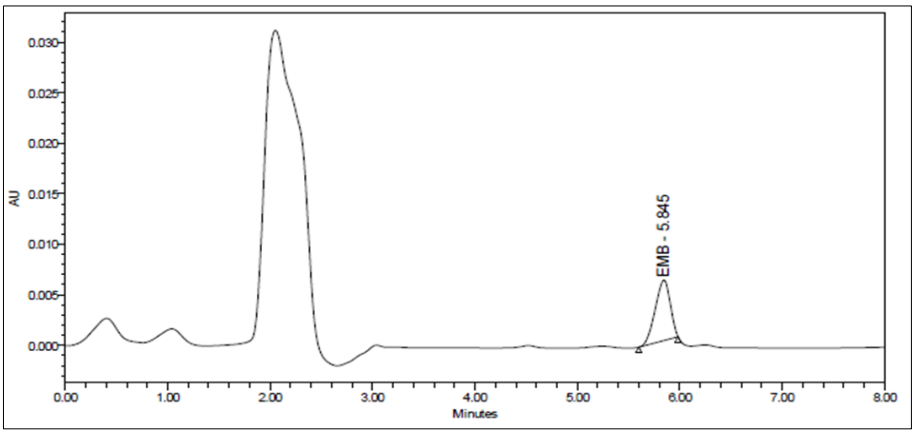
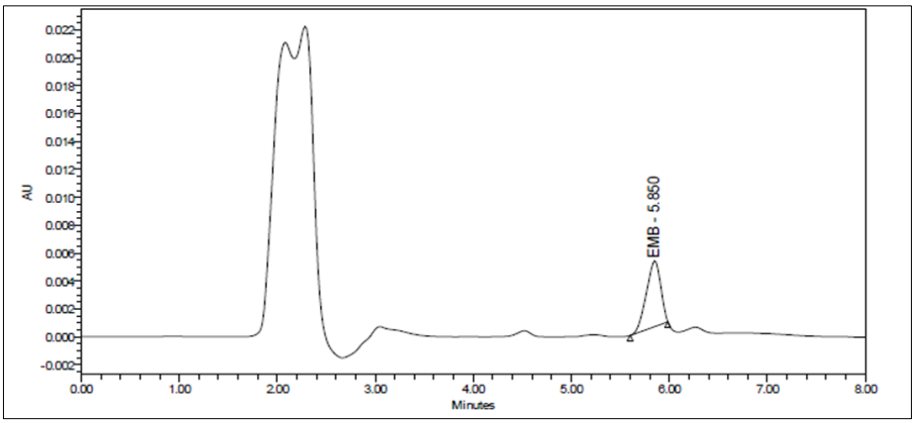
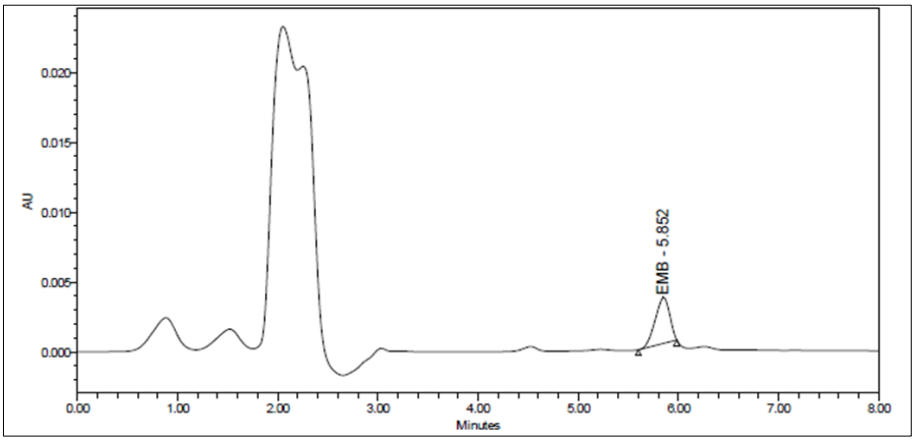


Fig 2: Plasma EMB concentration vs. time profiles after orally administration of EMB. Each time point (n = 3) represent the mean ± SE





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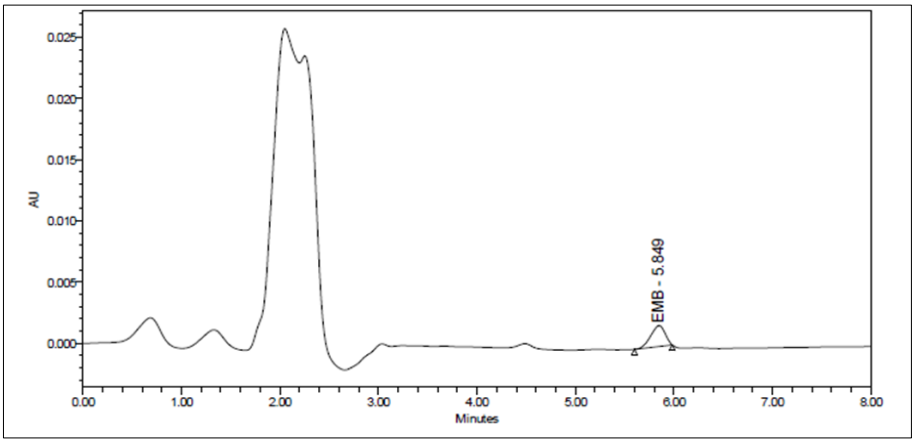
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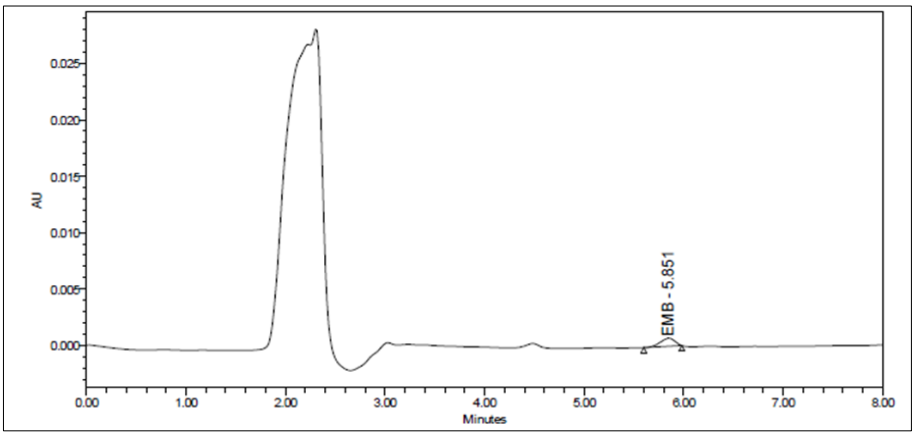
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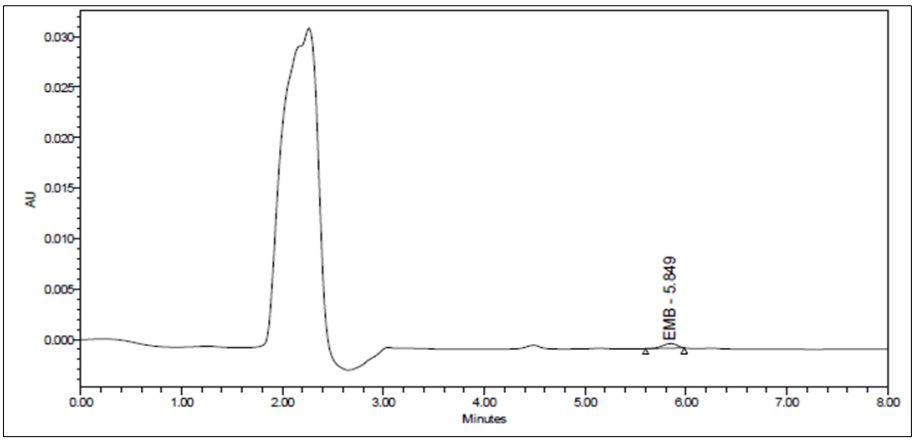
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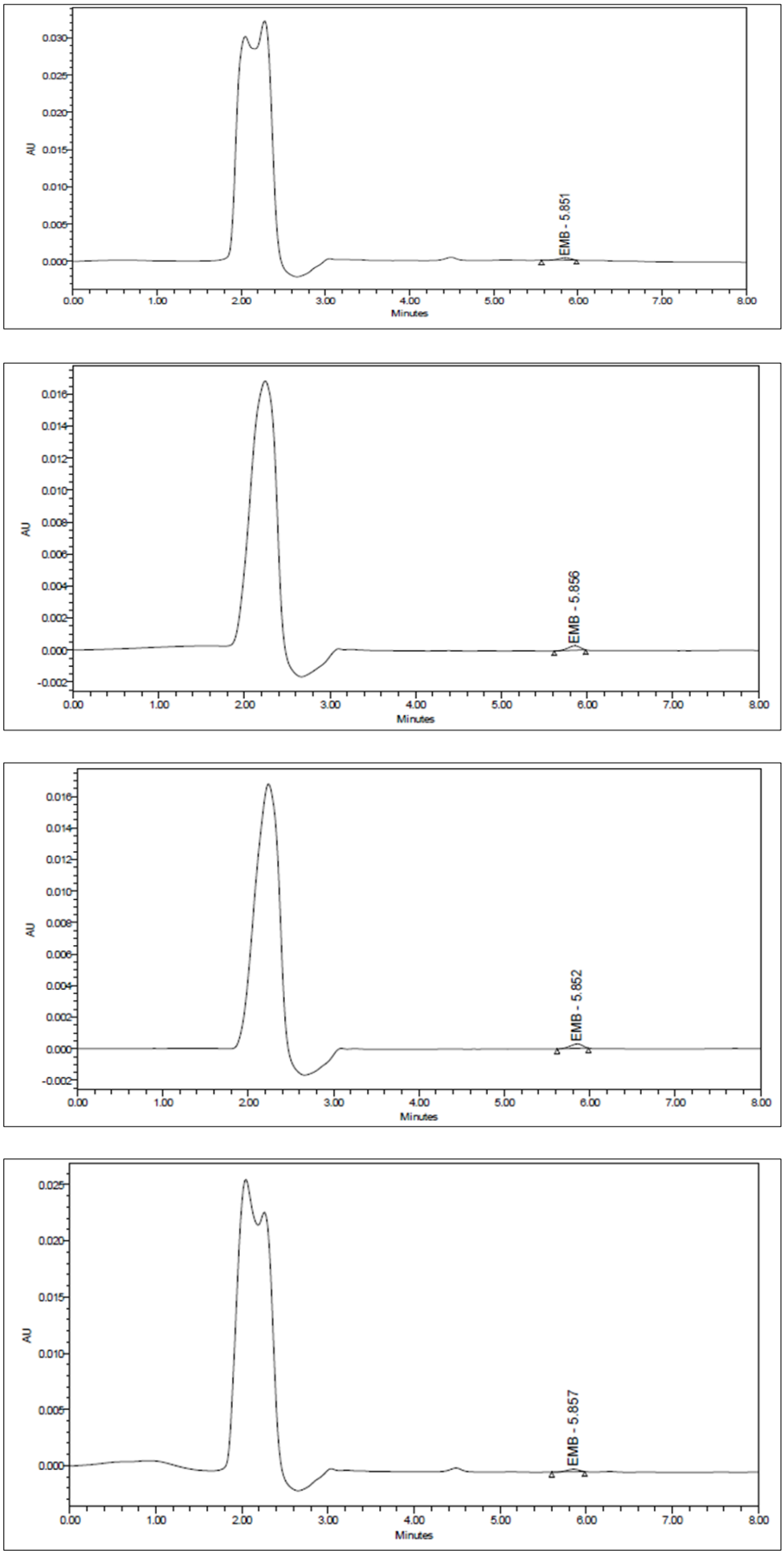


Fig 3: Chromatograph of EMB detected in plasma showing retention time 5.851 to 5.856 min at different time intervals (n = 3) A (0.5 h), B (1 h), C (2 h), D (4h), E (8 h), F (12 h), G (24 h), H (36 h), I (48 H), J (72 h) and K (96 H) postdose of drug administration

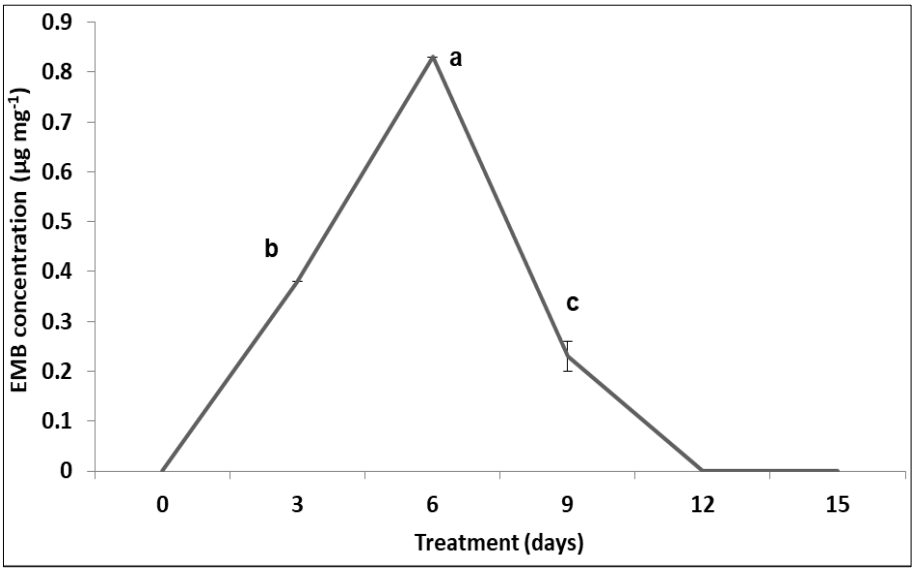
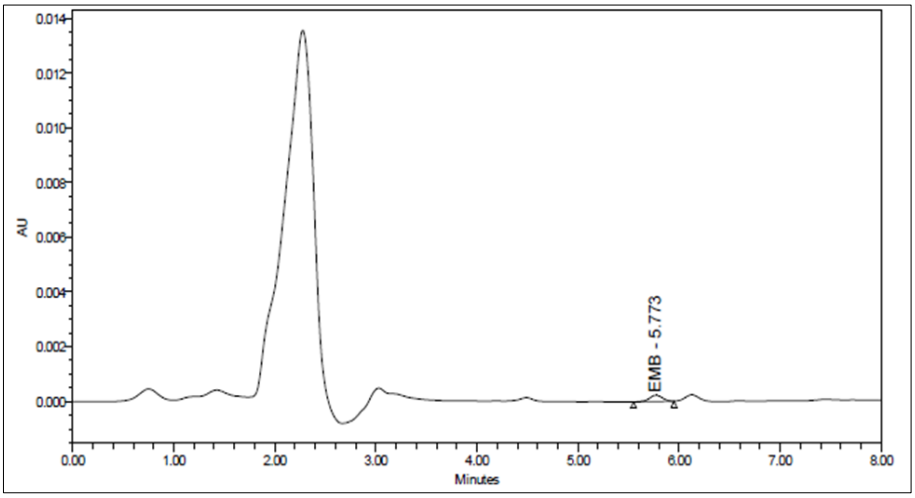
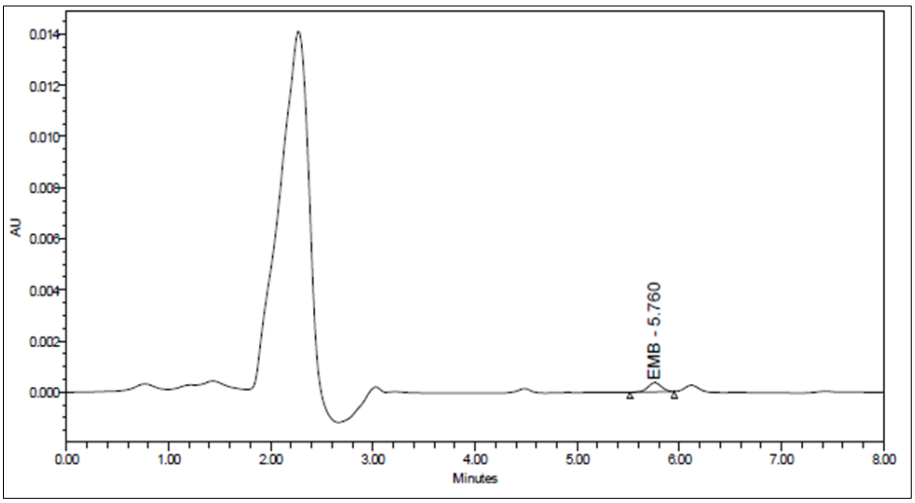


Fig 4: Muscle EMB concentration vs. time profiles after orally administration of EMB. Each time point (n = 3) represent the mean ± SE





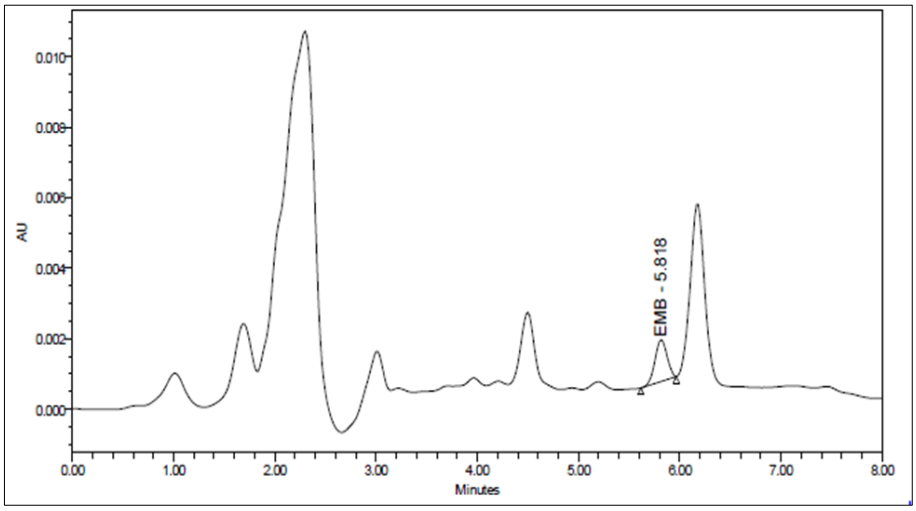


Fig 5: Chromatograph of EMB detected in muscle showing retention time 5.773 to 5.818 min at different time intervals (n = 3) A (3rd day), B (6th day), C (9th day), post dose of drug administration

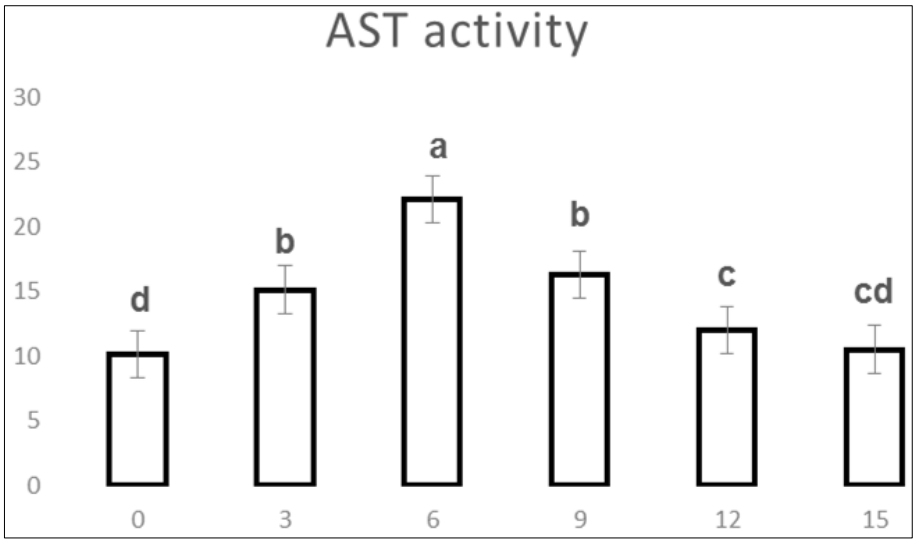


Fig 6: Aspartate amino transferase activity of *Labeo rohita* fingerlings fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as nanomoles oxaloacetate released/ min/ mg protein at 37°C, n=3, sampling intervals were 2 days

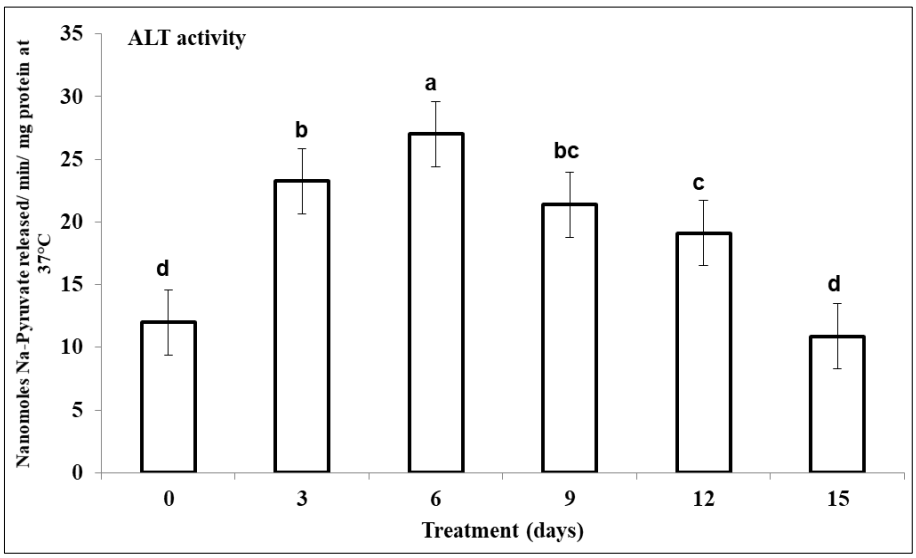


Fig 7: Alanine amino transferase activity of *Labeo rohita* fingerlings fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as Nanomoles Na-Pyruvate released/ min/ mg protein at 37°C, n=3, sampling intervals were 2 days

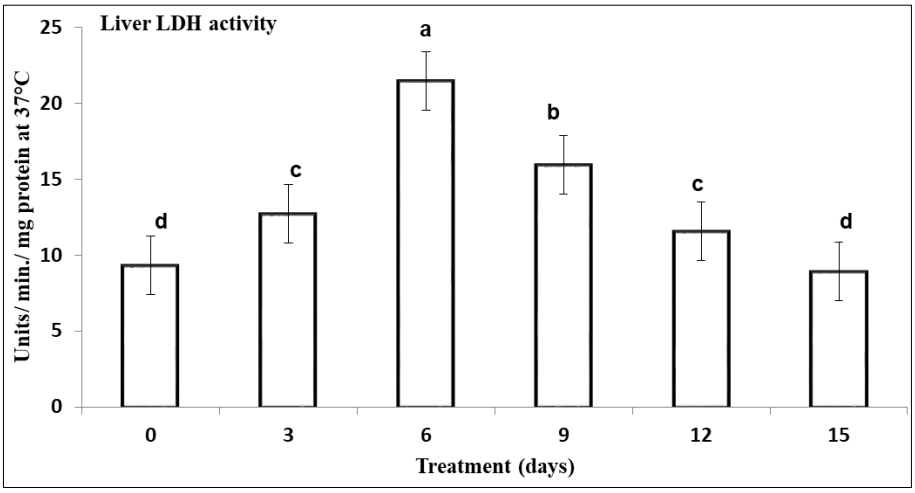


Fig 8: Lactate dehydrogenase activity of *L. rohita* fingerlings (liver) fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as Units/ min./ mg protein at 37°C, n=3, sampling intervals were 2 days

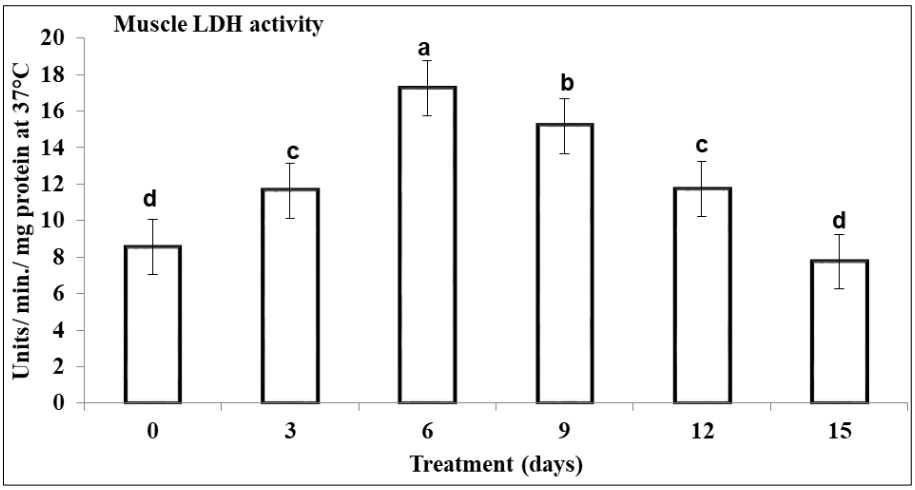


Fig 9: Lactate dehydrogenase activity of *Labeo rohita* fingerlings (muscle) fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as Units/ min./ mg protein at 37°C, n=3, sampling intervals were 2 days

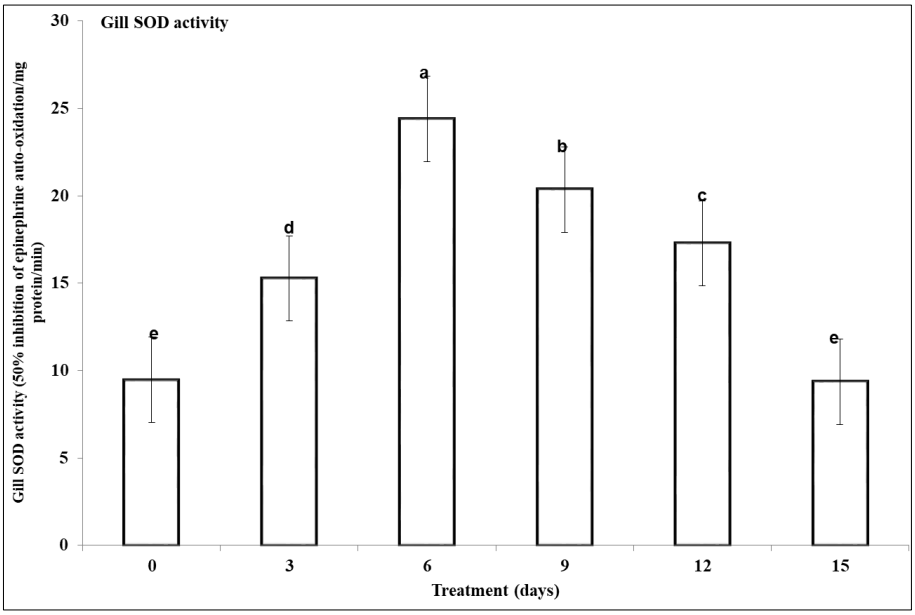


Fig 10: Superoxide dismutase activity of *L. rohita* fingerlings (gill) fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min, n=3, sampling intervals were 2 days

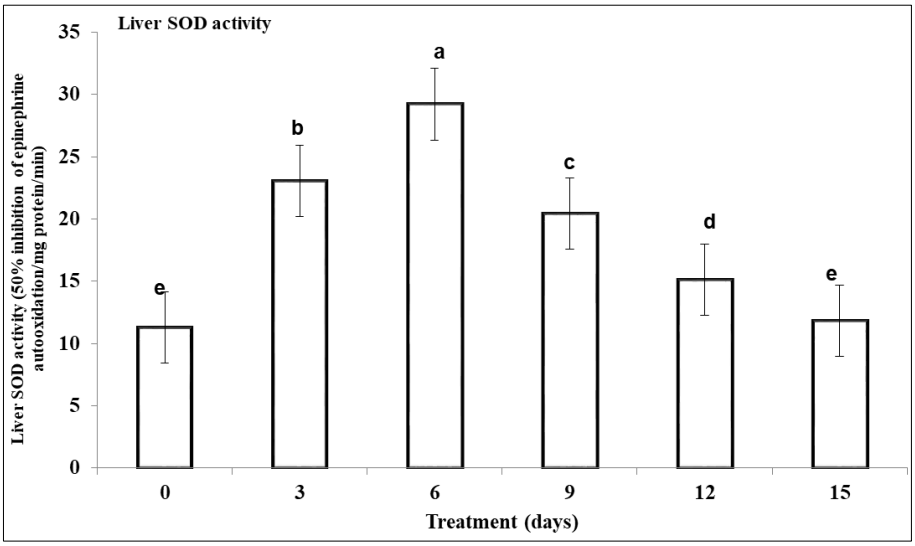


Fig 11: Superoxide dismutase activity of *L. rohita* fingerlings (liver) fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as 50% inhibition of epinephrine autooxidation/mg protein/min, n=3, sampling intervals were 2 days

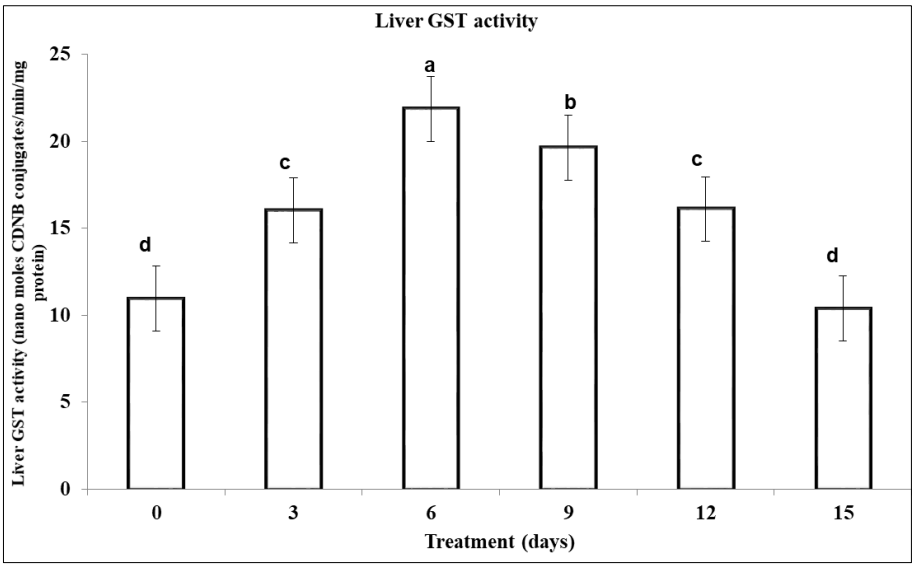


Fig 12: Glutathione S transferase activity of *L. rohita* fingerlings fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as nano moles CDNB conjugates/min/mg protein, n=3, sampling intervals were 2 days

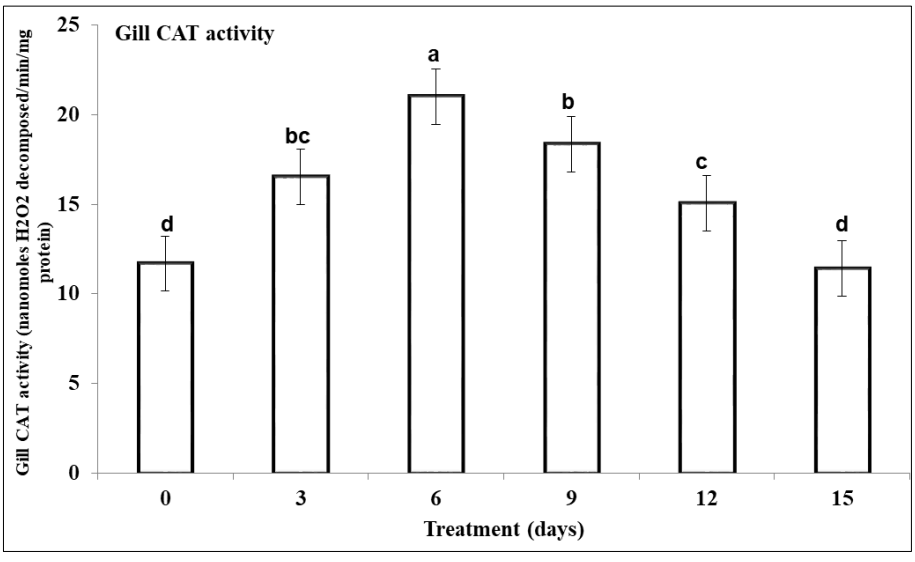


Fig 13: Catalase activity of *L. rohita* fingerlings (gill) fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as nanomoles of H_2O_2 decomposed/min/mg protein, n=3, sampling intervals were 2 days

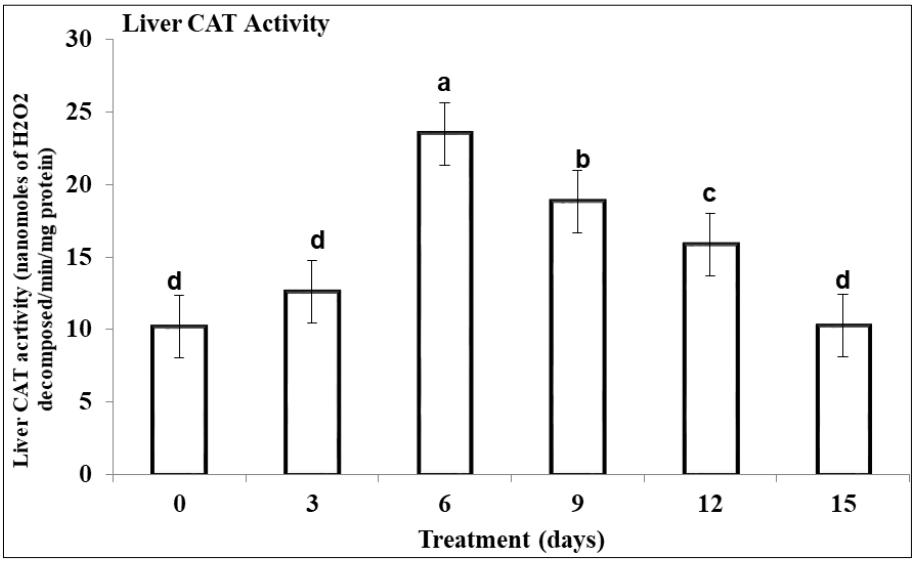


Fig 14: Catalase activity of *L. rohita* fingerlings (liver) fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as nanomoles of H_2O_2 decomposed/min/mg protein, n=3, sampling intervals were 2 days.

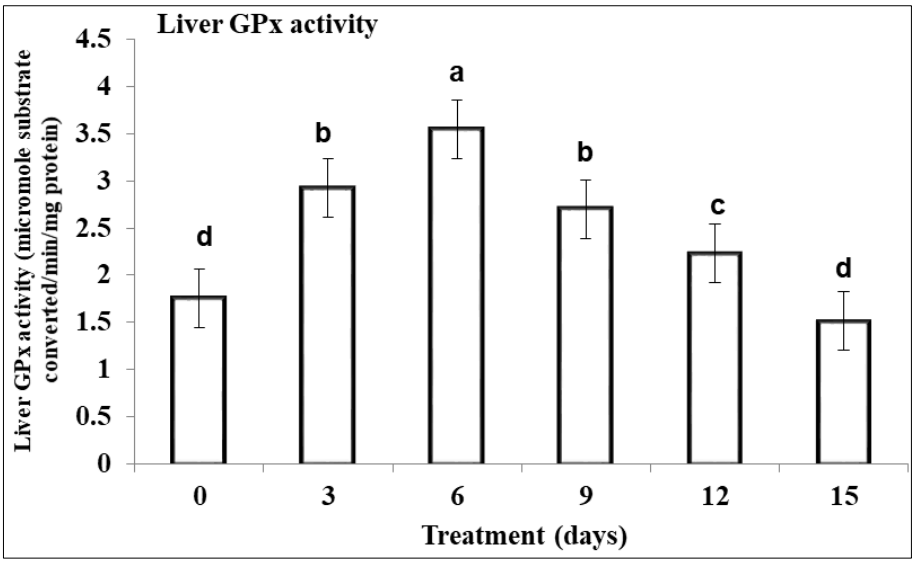


Fig 15: Glutathione peroxidase activity of *L. rohita* fingerlings fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as micromole substrate converted/min/mg protein, n=3, sampling intervals were 2 days

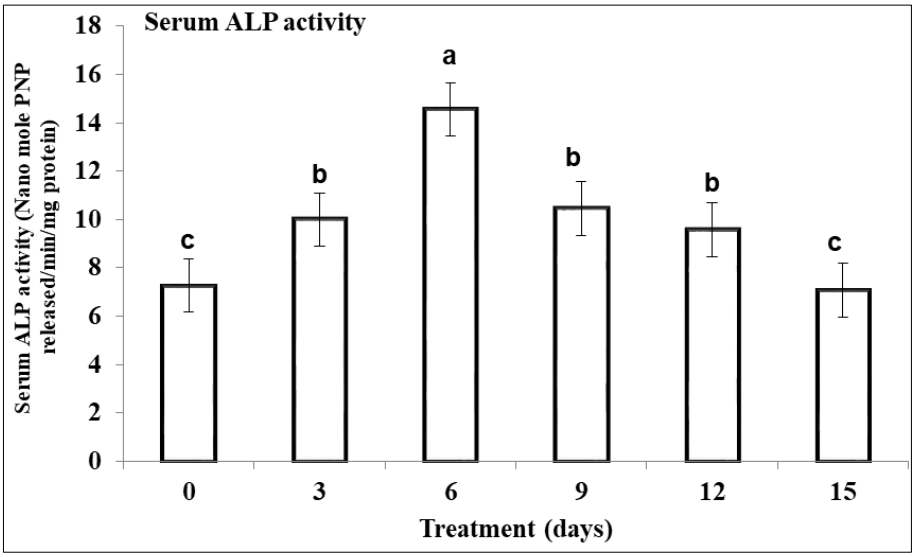


Fig 16: Alkaline phosphatase activity of *L. rohita* fingerlings fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as nano mole PNP released/min/mg protein, n=3, sampling intervals were 2 days

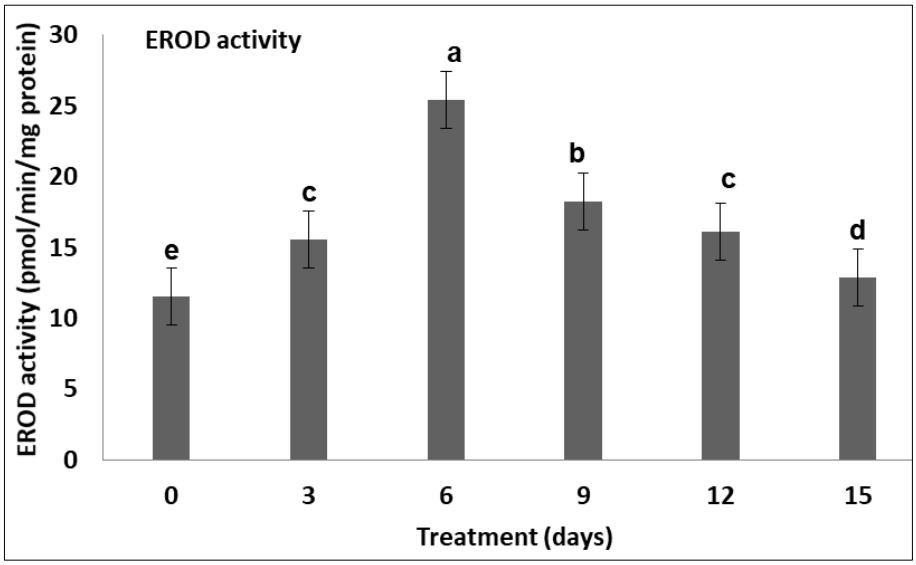


Fig 17: Ethoxyresorufin D ethylase activity of *L. rohita* fingerlings fed with EMB at the dose of 20mg/kg for 15 days. Data is expressed as pmol/min/mg protein, n=3, sampling intervals were 2 days

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