PHARMACOKINETICS AND PHYSIO-METABOLIC RESPONSES OF EMAMECTIN BENZOATE IN *LABEO ROHITA* (HAMILTON, 1822)

Dissertation submitted in partial fulfillment
of the requirements
for the degree of

M.F.Sc. (Fish Physiology and Biochemistry)

By

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DECLARATION

I hereby declare that the dissertation entitled **"PHARMACOKINETICS AND PHYSIO-METABOLIC RESPONSES OF EMAMECTIN BENZOATE IN *LABEO ROHITA* (HAMILTON, 1822)"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

Dated: 30th June, 2018. **(Diganta Chetia)**

Place: Mumbai M.F.Sc. Student

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Ministry of Agriculture, Govt. of India

Dated: 30th June, 2018

CERTIFICATE

Certified that the dissertation entitled **"PHARMACOKINETICS AND PHYSIO-METABOLIC RESPONSES OF EMAMECTIN BENZOATE IN *LABEO ROHITA* (HAMILTON, 1822)"** is a record of independent bonafide research work carried out by **Mr. Diganta Chetia** during the period of study from August 2017 to June, 2018 under our supervision and guidance for the degree of **Masters of Fisheries Science (Fish Physiology and Biochemistry)** and that the dissertation has not previously formed the basis for the awards of any degree, diploma, associateship, fellowship or any other similar title.

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DEDICATED TO,

MY BELOVED FAMILY AND GUIDE

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Abstract

Emamectin benzoate, 4”-deoxy-4”-methylamino derivative of abamectin, is a potential antiparasitic compound and widely used in animals and plants as herbicides. An experiment was conducted to evaluate the pharmacokinetics and physio-metabolic responses of emamectin benzoate in *Labeo rohita* (90±10). A preliminary study was conducted with oral administration of the drug at different doses viz 10, 20, and 50 mg kg^{-1} in relation to select an effective therapeutic dose. Blood samples were collected to examine the parent drug residue of EMB at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h of post-administration. HPLC was used for the determination of depletion profile of EMB in plasma using C-18 reversed phase analytical column (2.5mm×4.6mm×5.0µm) with a mobile phase of 0.1% orthophosphoric acid (H_3PO_4): Acetonitrile (ACN) at 60:40 and samples were measured at 244 nm by using a UV-visible detector. Depletion profile of EMB in plasma showed the drug residue detected maximum concentration (C_{max}) of 52.72 ± 0.02 µg ml^{-1} at 1 h (T_{max}) time point for 20 mg kg^{-1} body weight and 78.53 ± 0.01 µg ml^{-1} (C_{max}) at 1 h (T_{max}) for 50 mg kg^{-1} body weight. Biochemical analysis of tissues viz liver, gill, muscle, and serum revealed that the enzymes of ROS superfamily (SOD, CAT, GST, GPx) and metabolic enzyme (SGOT, SGPT, LDH) were significantly elevated in the 6 day and returned to a normal level by the 15 day of post-administration of EMB. Ethoxyresorufin-O-deethylase (EROD) analysis showed that the induction of CYP1A activity was 11.53 ± 0.46 to 25.44 ± 0.51 (pmol min^{-1} mg $protein^{-1}$) in rohu when administered with EMB through the feed. It is observed that the drug has induced anti-oxidant activity mechanisms that would be subsided the oxidative and metabolic stress in experimental fish within two weeks. The study evident that fish recovered stress within 15 days after oral administration of EMB at 20 mg kg^{-1} BW.

सारांश

इमामेक्टिन बेन्ज़ोएट, एबामेक्टिन का एक व्यूत्पन्न, कृमिनाशक यैगिक है, जिसका उपयोग प्राय: जानवर में किया जाता है, इसके आलावा यह एक खर पतवार से रूप में भी प्रयोग होता है। लैबियो रोहिता (90 ± 10) में एमेमेक्टिन बेंजोएट के फार्माकोकेनेटिक्स और कायिकी-चयापचय प्रतिक्रियाओं का मूल्यांकन करने के लिए एक प्रयोग आयोजित किया गया था। एक प्रभावी चिकित्सकीय खुराक चुनने के संबंध में दवा के मौखिक प्रशासन के साथ 10, 20, और 50 मिलीग्राम किग्रा $^{-1}$ के साथ एक प्रारंभिक अध्ययन आयोजित किया गया था। 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, और प्रशासन के 168 h पर EMB के मूल दवा अवशेष की जांच के लिए रक्त के नमूने एकत्र किए गए थे। HPLC का उपयोग 0.1% ऑर्थोफॉस्फोरिक एसिड (H_3PO_4) के मोबाइल चरण के साथ सी -18 रिवर्सड चरण विश्लेषणात्मक कॉलम (2.5 मिमी × 4.6 मिमी × 5.0μm) का उपयोग करके प्लाज्मा में EMB की कमी प्रोफ़ाइल के निर्धारण के लिए किया गया था, 60 पर एसीटोनिट्रियल (ACN) : 40 और नमूने को यूवी-दृश्य डिटेक्टर का उपयोग करके 244 nm पर मापा गया था। प्लाज्मा में EMB की विलोपन प्रोफाइल ने दिखाया कि दवा अवशेष में 52.72 ± 0.02 μg ml $^{-1}$ का अधिकतम सांद्रता (C_{max}) 1 h (T_{max}) समय बिंदु 20 मिलीग्राम किलो $^{-1}$ शरीर वजन और 78.53 ± 0.01 μg ml $^{-1}$ (C_{max})) 50 मिलीग्राम किलो $^{-1}$ शरीर वजन के लिए 1 h (T_{max}) पर। यकृत, जैल, मांसपेशियों और सीरम जैसे ऊतकों के जैव रासायनिक विश्लेषण से पता चला कि ROS सुपरफैमिली (SOD,CAT,GST,GPx) और चयापचय एंजाइम (SGOT, SGPT, LDH) के एंजाइमों को 6 दिनों में काफी बढ़ाया गया था और वे वापस लौटे ईएमबी के बाद प्रशासन के 15 दिन तक सामान्य स्तर। इथॉक्सीरेसोरफिन-ओ-डेथिलेज़ (EROD) विश्लेषण से पता चला है कि CYP1A गतिविधि की प्रेरण 11.53 ± 0.46 से 25.44 ± 0.51 (pmol min $^{-1}$ मिलीग्राम प्रोटीन $^{-1}$) रोहू में होती है जब फ़ीड के माध्यम से ईएमबी के साथ प्रशासित होता है। यह देखा गया है कि दवा ने एंटी-ऑक्सीडेंट गतिविधि तंत्र प्रेरित किया है जो दो सप्ताह के भीतर प्रयोगात्मक मछली में ऑक्सीडेटिव और चयापचय तनाव से कम हो जाएगा। अध्ययन से पता चला है कि 20 मिलीग्राम किलो $^{-1}$ BW पर EMB के मौखिक प्रशासन के 15 दिनों के भीतर मछली ने तनाव को पुनर्प्राप्त किया।

CONTENTS

1. INTRODUCTION

The expansion and intensification of the farming operation is the necessity of the present day aquaculture to ensure food security to the growing population of India. The 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 & Sahoo, 2006). Rohu (*Labeo rohita*) is an economically important freshwater fish species of the Indian subcontinent, which contributes to about 6.5% and 35% of the world's and India's total carp production respectively (FAO 2008). *Labeo rohita* is one among the Indian major carps which contribute to 35% of total carp production in the world. This species is intrinsically sensitive to various stressors in intensive and semi-intensive systems and it was found that it is more susceptible to disease than exotic carps (Lilley *et al.,* 1992). The infectious agents causing diseases in the stressful environment mainly include parasites, bacteria, and virus. Among the most common causative agents of infectious diseases in aquaculture are bacteria (54.9%), followed by viruses (22.6%), parasites (19.4%) and fungi (3.1%).

Parasites are one of the major groups of organisms that may or may not cause infection in fishes depending on a number of factors. Parasites in fish (endo and ectoparasites) are a natural occurrence and common (Moyle *et al.,* 2004). Fish parasites result in huge economic losses as they increase mortality and also increase farm inputs via increased treatment expenses and cause a reduction in growth rate due to the parasitic disease outbreak (Kayis *et al.,* 2009). The loss due to an infestation of parasites in *Labeo rohita* has been reported and the highest prevalence of infection (94.54%) in *L. rohita* was found to be in winter (Monir *et al.,* 2015). The overall loss due to parasitic diseases was found 11% for mortality, 11% for chemicals cost and 65% for the reduction of growth. The intensity of parasitic infection in fish is greatly influenced by seasonality, which affects host ecology as well as physiology (Pennycuick *et al.,* 1972; Rahman *et al.,* 2007). Intense parasitic infection can cause ulceration and upset the normal course of reproduction (Rahman *et al.,* 2005). Parasites interfere with host nutrition, metabolism and secretary functions of the alimentary canal and can even

damage the host nervous system (Markov *et al.,* 1961). All these effects may reduce the normal growth of the fish and finally result in the host mortality.

Protozoans are the most common parasites encountered in fish hatcheries (Pillay, 1995). Among the protozoan parasites, *Ichthyobodo necator*, *Ichthyophthirius multifilis, Trichodina* spp., *Trichophyra, Ambiphyra, Hexamita*, and *Apiosoma* are some of the most significant pathogens in aquaculture (Durborow, 2003) and their prevention methods are still in infant stage. Several instances of argulosis, a crustacean parasitic disease, associated with fish mortality have also been reported from cultured ponds (Gopalakrishnan 1964; Singhal *et al.,* 1990; Sheila *et al.,* 2002). The management and control of parasitic infections in aquaculture are a constant challenge, highly complicated by the current limited availability of efficacious licensed products, a situation exacerbated by the development of resistance to antiparasitic drugs in parasite populations. In addition, parasite control in aquaculture requires a keen awareness of environmental, water quality and host parameters, and is subject to the constraints of economics and the requirement for aquaculture sustainability and environmental protection. There are a number of chemotherapeutics used or proposed against parasitic infestation in fishes. Emamectin benzoate (4″-deoxy-4″ methylamino derivative of abamectin) is widely used in controlling *lepidopterous* pests in agricultural products in the US, Japan, Canada, and recently in Taiwan (Yen and Lin, 2004). Emamectin has also been successfully employed by fish farmers to control the sea lice in Atlantic salmon (Ikeda and Omura, 1997; Rodriguez *et al.,* 2007). The countries such as United Kingdom, Chile, Ireland, Iceland, Finland, the Faroe Islands, Spain, and Norway are currently registered to use emamectin in their fish feed (Ikeda and Omura, 1997). Removal of the afflicting sea louse represents an increase in the quality of their salmonid product due to the subsequent reduction of bacterial and viral pathogens possibly carried by the sea lice. Emamectin has shown efficacy against all life-cycle stages of *Lepeophtheirus salmonis* (Salmon louse) and *Caligus elongatus* (Sea louse), (Rodriguez *et al.,* 2007).

In view of the absence of information on the pharmacokinetic study of emamectin in *Labeo rohita,* the present study may reveal the pharmacokinetics of

emamectin in *Labeo rohita* and physio-metabolic changes in *Labeo rohita* when fed with medicated feed containing emamectin benzoate. In this background, the study was designed with the following objectives.

1. Pharmacokinetics study of emamectin benzoate in *Labeo rohita*.

2. To study physio-metabolic responses in *Labeo rohita* treated with emamectin benzoate through the feed.

2. REVIEW OF LITERATURE

Parasitic infections are very common in aquatic ecosystem and as a result of this infection with the complex interaction of many interrelated factors including parasite population dynamics and the presence of other diseases causing agents results in host mortality in carps. Fish skin and gills are the habitats of many ectoparasites. The most common are monogeneans and certain groups of parasitic copepods, which can be extremely numerous in a number of the host animal (Kearn, 2005).

2.1. Chemotherapeutants used or proposed for the treatment of parasite infestation

2.1.1. Chemotherapeutants for ectoparasites

For treatment of protozoan infections formalin (Speare *et al.,* 1997), hydrogen peroxide (Derksen *et al.,* 1999; Itou *et al.,* 1997), chloramine-T (Meinertz *et al.,* 1999), potassium permanganate (Flores-Crespo *et al.,* 1995), copper sulphate (Cardeilhac and Whitaker, 1988),iodophors (Evelyn *et al.,* 1986), malachite green (Alderman, 1982) are widely used as bath treatment. Benzimidazoles (Manger, 1991), levamisole (Anderson, 1992), niclosamide (James and Gilles, 1985) and bithionol (James and Gilles, 1985) are used as a bath treatment for monogenean infection. Organophosphates (Burka *et al.,* 1997), pyrethrins and pyrethroids (Blagburn and Lindsay, 1995), chitin synthesis inhibitors (Ritchie *et al.,* 2002), avermectins and related drugs (Burka *et al.,* 1997), are used to cure copepod infection.

2.1.2. Chemotherapeutants for endoparasites

The chemotherapeutic agent used for the treatment of endoparasitic infection are fumagillin (Shadduck, 1980), TNP-470 (Jaronski, 1972),toltrazuril (Mehlhorn *et al.,* 1984), amprolium (Tojo and Santamaria, 1998a, 1998b, 1998c), quinine (Schmahl *et al.,* 1996), metronidazole (Tally and Sullivan, 1981; Lau *et al.,*

1992), salinomycin (Kinashi *et al.,*1973), benzimidazoles (Schmahl and Benini, 1998),sulphonamides (Alderman, 1988), and oregano oils (Daferera *et al.,* 2000).

2.2. Emamectin benzoate (EMB)

Emamectin is the 4”-deoxy-4”-methylamino derivative of abamectin, a 16-membered macrocyclic lactone produced by the fermentation of the soil actinomycete *Streptomyces avermitilis* (Kaoukhov and Cousin, 2009, Grossman and Cox, 2010). It is generally prepared as the salt with a benzoic acid, emamectin benzoate, which is a white or faintly yellow powder (Waddy *et al.,* 2007). Emamectin is widely used in the US and Canada as an insecticide because of its chloride channel activation properties (Andersch *et al.,* 2011). EMB has broad spectrum of activity against parasitic infections like *Caligus curtus* (Hamre *et al.,* 2011), sea lice (Skilbrei *et al.,* 2008), *Lepidopterus* pest (Jansson *et al.,* 1997), *Lepeophtheirus salmonis* (Stone *et al.,* 2002; Lees *et al.,* 2008; Saksida *et al.,* 2010; Igboeli *et al.,* 2012; Whyte *et al.,* 2013; Helgesen *et al.,* 2015), *Larnanthropus kroyeri* (Toksen *et al.,* 2006), *Anguillicoloides crassus* (Larrat *et al.,* 2012) *Argulus siamemsis* (Kar *et al.,* 2015), *Argulus coregoni* (Hakalahti *et al.,* 2004), and *Caligus rogercresseyi* (Bravo *et al.,* 2008).

2.2.1 Chemical structure and nomenclature of emamectin benzoate

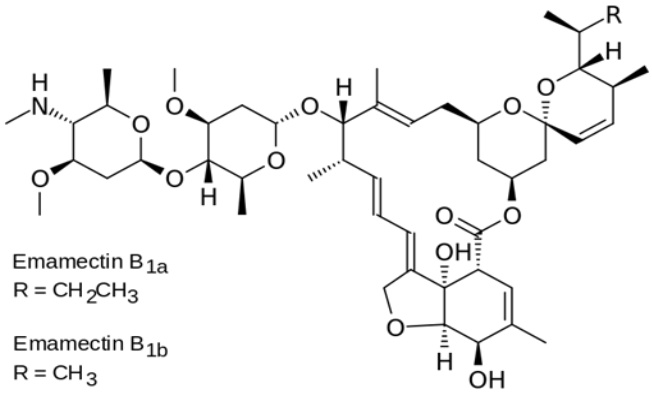
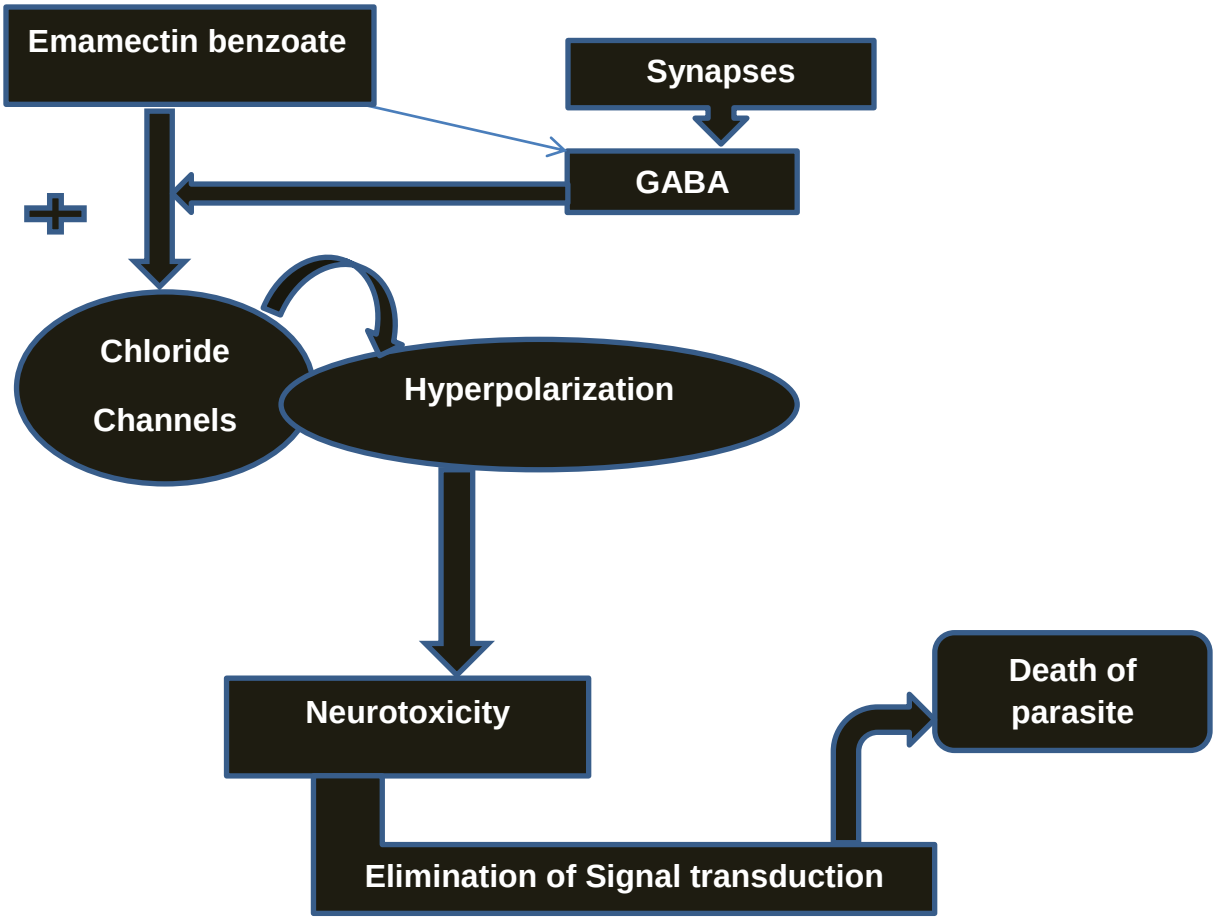


Table. 1. Properties of emamectin benzoate

Chemical formula	$C_{49}H_{75}NO_{13}$
Molar mass	886.13 g·mol^{-1}
Appearance	White or faintly yellow powder
Melting point	141 to 146 °C (286 to 295 °F; 414 to 419 K)
Solubility in water	30-50 ppm (pH 7)
Other names	4″-Deoxy-4″-epi-methylamino avermectinB1; Epimethylamino-4″-deoxy avermectin; MK 243; EMA; GWN 1972

2.2.2. Mode of action of emamectin

Emamectin works as a chloride channel activator by binding gamma-aminobutyric acid (GABA) receptor and glutamate-gated chloride channels disrupting nerve signals within arthropods (Grant, 2002; Atlanta GA: USDA/Forest Service. 2008). The compound stimulates the release of GABA from the synapses between nerve cells and while additionally increasing GABA's affinity for its receptor on the post-junction membrane of muscle cells in insects and arthropods (Rodriguez *et al.,* 2007). The stronger binding of GABA increases the permeability of the cell to chloride ions within the cell due to the hypotonic concentration gradient (Rodriguez *et al.,* 2007). Neurotransmission is thereby reduced by subsequent hyperpolarization and the elimination of signal transduction (Rodriguez *et al.,* 2007).



2.2.3. Emamectin as an anti-parasitic drug

Skilbrei *et al.,* (2015) evaluated the efficacy of abamectin benzoate and substance EX against sea lice in Atlantic salmon smolts. EMB was administered to salmon smolts in high doses by intraperitoneal injection and the prophylactic substance EX (SubEX) was administered by bathing. They identified that both treatments appeared to give the smolts a high degree of protection against infestation of salmon lice (copepods). Another study on the efficacy of emamectin benzoate as an oral treatment against, *Lepeophtheirus salmonis* (sea lice) infestations in Atlantic salmon, (*Salmo salar*) was carried out by Stone *et al.,* (1999). The Emamectin benzoate was given through pelleted feed at doses of 0, 25, 50 and 100 µg kg^{-1} $biomass^{-1}day^{-1}$ for seven consecutive days and found the total numbers of sea lice were significantly

reduced at all doses when compared to control fish. Hakalahti *et al.,* (2004) have studied the efficacy of dietary emamectin benzoate in the control of *Argulus coregoni* infestation in *Oncorhynchus mykiss*. Efficacy of 100% against newly hatched *Argulus coregoni* metanauplii and adults and 80% of juveniles were observed. Bravo *et al.,* (2008) have reported the sensitivity assessment of sea louse (*Caligus rogercresseyi*) to emamectin benzoate in salmon. Lees *et al.,* (2008) studied the efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon in Scotland, and they found that sea lice infestations are reduced following the application of emamectin benzoate, not all treatments are effective. Stone *et al.,* (2002) have reported the safety and efficacy of emamectin benzoate, administered through the feed to Atlantic salmon in freshwater as a preventive treatment against sea lice *Lepeophtheirus salmonis.*

2.2.4. Treatment dose in medicated feed

In cultured sea bass, emamectin benzoate appears to be highly effective for the treatment of *Lernathropus kroyeri,* a copepod infestation (Toksen *et al.,* 2006). The number of parasites had decreased in the treated fish by 55%, 50%, 43%, and 74% at the dose of 10, 25, 50, and 100 µg kg^{-1}, respectively. EMB was effective in the control of *Caligus curtus* infestations in Atlantic cod, *Gadus morhua* (Hamre *et al.,* 2011), following an administration of 50 µg kg^{-1} for seven consecutive days. Stone *et al.,* (1999) found that emamectin benzoate at 50 µg kg^{-1} dose resulted in significantly fewer lice than the 25 µg kg^{-1} dose. The optimum therapeutic dose was selected as 50 µg $kg^{-1}day^{-1}$ for seven days. Hakalahti *et al.,* (2004) reported that administration of emamectin benzoate at 50 µg $kg^{-1}day^{-1}$ through feed for 7 days can control *Argulus coregoni* infestation in *Oncorhynchus mykiss.*

2.2.5. Pharmacokinetics of emamectin

A study conducted by Samuelsen (2010) revealed the pharmacokinetic profile of the antiparasitic agent emamectin benzoate in plasma after intravenous injection and in plasma, muscle, and skin following oral administration to cod held in seawater at 9^0C the plasma distribution half-life (t1/2α) was estimated at 2.5 h, the

elimination half-life (t1/2β) as 216 h, and the total body clearance as 0.0059 L kg^{-1} h^{-1} and mean residence time (MRT) as 385 h.

2.2.6. Effect and toxicity of EMB in fish

Signs of emamectin benzoate (EMB) toxicity in fish included lethargy, dark coloration, and anorexia. Atlantic salmon showed loss of coordination. Fish exposed to high dose of EMB exhibited signs of toxicity and showed no evidence of recovery during the 7 day post-treatment period. No pathognomonic signs of emamectin benzoate toxicity were identified during gross necropsy or histopathological examination. *Roy et al.,* (2000) studied the tolerance of Atlantic salmon, *Salmo salar L*., and rainbow trout, *Oncorhynchus mykiss* to emamectin benzoate, a new orally administered treatment for sea lice for 7 days. No mortality was observed, which could be related to treatment and unequivocal signs of toxicity which recorded only at the highest dose used in these studies. Roy *et al.,* (2000) conducted laboratory studies with both *Salmo salar* and *Oncorhynchus mykiss.* The study concluded that Atlantic salmon could tolerate EMB at doses up to 173 µg kg^{-1} BW day^{-1} (3.4x recommended Slice dose) and that toxic effects were observed only at doses of 356 µg kg^{-1} BW day^{-1} (7.1x recommended dose). A study conducted in *O. mykiss* reported that the fish can tolerate EMB at doses up to 218 µg kg^{-1} BW day^{-1} and that toxicity effects were observed at doses of 413 µg kg^{-1} BW day^{-1}.

The Office of Pesticide Programs conducted a study in 2000, wherein the researchers exposed sheepshead minnow (*Cyprinodon variegates*) to EMB in a flow-through system for 96 h. The medium lethal concentration (LC_{50}) in seawater was 1.43 ppm, with a range of 1.25 ppm to 1.67 ppm. Similarly, bluegill sunfish (*Lepomis macrochirus*), rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) average LC_{50} values of 180, 174 and 194 ppb, respectively, were observed.

2.2.7. Excretion of emamectin

Although excretion rates are not used directly in either the dose-response assessment or risk characterization, excretion half-lives can be used to infer the effect

of longer-term 22 exposures on body burden, based on the plateau principle (Goldstein *et al.,* 1974).

2.2.8. Withdrawal period and residual level

A radiolabelled study in salmon demonstrated that emamectin B1a (a derivative of EMB) is the marker for residue accumulation as it remains predominantly unmetabolized (Kim-Kang *et al.,* 2004). Over the time interval between the last administration and 70 days post-administration, the ratio between the marker residue and the total radioactive residue (expressed as emamectin benzoate equivalents) was stable in muscle and skin, at 65 ±7% for both tissues. After correction, the ratio of the mean concentration of the marker residue emamectin B1a to that of the total residue was calculated as 0.9 µg kg^{-1} for muscle and fillet (muscle+skin), and 0.8 µg kg^{-1} for the skin. Residues decline slowly in fish and terminal half-lives are dependent on environmental conditions. The recommended Maximum Residue Limits (MRLs) of 100 µg kg^{-1} of emamectin in muscle and fillet (muscle+skin) are based on the upper limit of the one-sided 99% confidence interval over the 99 percentile (99/99 tolerance limit) for the 10-day post-treatment of the radiolabelled depletion curve. This highest value 99/99 instead of 95/95 was chosen by the committee to cover the uncertainty associated with the high terminal half-life and the variation of kinetics in fish in relation to living conditions. Data on residue accumulations were obtained using a validated analytical method to quantify emamectin B1a in tissue. Residue data from two independent studies were also provided for trout administered unlabeled emamectin benzoate at the approved dose rate. Median concentrations in muscle and fillet reported in trout were in the same range as those observed in salmon (Roy *et al.,* 2006).

2.2.9. Environmental risk

In the laboratory studies with marine sediments and seawater, only two to three percent of the EMB was recovered from the seawater, with a similar proportion being recovered in the water following desorption phase from sediments. It has been determined that up to 5% of EMB can leach off from the medicated feed over a six-hour period, and approximately 25% after 7 days, following shaking in seawater for 5 minutes

(SPAH, 2002). Davies *et al.,* (1997) determined that less than 5% of ivermectin leached off medicated feed over a 48 h period and that its physicochemical properties suggest that leached ivermectin would adsorb onto surrounding sediments. Field studies involving silt traps adjacent to fish cages showed that about 1% of the total EMB in the traps was in the water phase. This material may consist of both soluble and fine particle-associated material (SPAH, 2002).

EMB in a soluble form in water may arise by equilibration from the sediment-bound material into interstitial water, and then potentially into overlying waters. This action, assuming that input mechanisms are no longer active, has the potential to dilute the sediment concentrations over time. This is supported by adsorption/desorption and marine degradation studies which revealed that residual levels of EMB were found in the seawater phase throughout the study. This was further supported by reports that EMB did not significantly accumulate in the sediments, despite being detected in settling material (SPAH, 2002).

Gavage feeding of radio-labeled EMB to salmon and subsequent analysis revealed that higher proportions of metabolites were found in gut contents at all-time points that those found in tissue samples. This data indicates that the metabolites are excreted more rapidly than the parent compound, or that the parent compound is subject to more enterohepatic circulation than the metabolites (SPAH, 2002). Similar studies involving variations in water temperature revealed that when temperatures were 10˚C, almost all of the excreted material was metabolites, whereas only 30% of the excreted material (from treatment to 90 days post-treatment) was metabolites at 5˚C (SPAH, 2002). Kim-Kang *et al.,* (2004) administered radiolabeled emamectin benzoate EB to *S. salar,* maintained at 5˚C (+/- 1˚C), and collected tissue, blood, and bile from fish at 3 and 12 hours, and 1, 3, 7, 15, 30, 45, 60 and 90 days post-treatment (final dose). Feces were also collected daily from the tank and monitored for total radioactive residues (TRR). The residue components of the liver, kidney, muscle, and skin samples pooled by postdose interval were emamectin B1a (81-100% TRR) and desmethylemamectin B1a (0-17% TRR) with N-formylemamectin B1a seen in trace amounts (<2%) in some muscle samples.

Bioaccumulation is the term describing a process whereby a substance is accumulated by organisms directly from the surrounding media and through consumption of food containing the substances. Bio-concentration is a process whereby there is a net accumulation of a substance directly from water into aquatic organisms resulting from simultaneous uptake (e.g., gills or epithelial tissue) and elimination. In the categorization process, bioaccumulation factors (BAF) are preferred over bio-concentration factors (BCF), however, in the absence of BAF or BCF data, the octanol-water partition coefficient (log KOW) may be used. The octanol-water partition coefficient (log KOW) is the ratio of the concentration of a material in the octanol phase to the concentration in the aqueous phase of a two-phase octanol/water system (Environment Canada, 2004).

3. MATERIALS AND METHODS

3.1. The site of the experiment

The experiment was conducted over a period of 31 days from 1st March to 31st March 2018 at the Fish Nutrition, Biochemistry and Physiology, wet laboratory of the ICAR-Central Institute of Fisheries Education (CIFE), Seven Bungalows campus Mumbai. The HPLC analysis and biochemical analysis were carried out in the biochemistry and the nutrition laboratories of Fish Nutrition, Biochemistry and Physiology, division of CIFE.

3.2. Experimental animals

Animals used for the experimental purpose were juveniles of rohu, *Labeo rohita* with an average weight of 90 ± 10 g. The fishes were procured from Arrey fish farm, Goregaon, Mumbai (Maharashtra) during the month of December. The fishes were transported in a big circular tank (500 L) with sufficient aeration to the old campus. They were carefully transferred to a circular FRP tank and were left undisturbed the whole night. In order to ameliorate the handling stress the fishes were given a mild salt and $KMnO_4$ treatment on the next day. The stock was acclimatized under the aerated condition for a period of two months. All fishes were found to exhibit normal behavior throughout the experimental duration.

3.3. Experimental setup

The animal experiment was conducted for a period of one month on the old campus of ICAR-Central Institute of Fisheries Education, Mumbai. The setup consisted of 27 plastic tanks of 200 L capacity covered with perforated lids. The tanks were initially washed with potassium permanganate solution (4 mg L^{-1}) that was left overnight. The tanks were flushed out the next day and were thoroughly washed with clean water. One hundred sixty-two healthy juveniles of *Labeo rohita* were randomly distributed in 27 experimental tanks with six fishes in each tank. The total volume of the water in each tank was maintained at 190 L throughout the experimental period. Round

the clock aeration was provided. The aeration pipe in each tub was provided with an air stone and a plastic regulator to control the air pressure uniformly in all the tanks.

3.4. Experimental design

One hundred sixty-two healthy juveniles of *Labeo rohita* were randomly distributed in 27 experimental tanks with six fishes in each tank. Among them 27 tanks received three doses of EMB (9 tanks for one dose like 10 mg kg^{-1} body weight, 20 mg kg^{-1} body weight, and 50 mg kg^{-1} body weight), one tank was kept as control and did not receive any treatment. Sampling was done on the 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h. For biochemical analysis, 28 fishes were randomly distributed in 4 tanks. Seven fishes were kept in each tank. Twenty-one fishes, together from 3 tanks received a single dose of EMB @ 20 mg kg^{-1} BW through the feed and the samples were collected on 0, 3, 6, 9, 12, and 15 days of post administered. At each sampling time point, three fishes were sacrificed.

3.5. Rearing

Fishes of uniform size were kept in a large circular tank (500 L). The tank was covered with a perforated lid to prevent the animals from jumping out. The fishes were fed a control diet throughout the experiment. No attempt was made to stimulate or control the environmental condition. The fishes are given formalin treatment at 25 ppm at regular intervals in order to remove the ectoparasites. The experimental conditions were kept same throughout the experiment.

3.6. Cleaning and siphoning

The experimental tanks were cleaned by manual siphoning every day in order to remove the excess feed pellets and remaining fecal matter. An equal volume of clean bore well water replaced the siphoned water. This cleaning was carried out throughout the experimental period of 30 days.

3.7. Control diets

A purified diet of crude protein 32.09% and lipid 6% were prepared by using purified ingredients such as casein, gelatin, dextrin, starch soluble, cellulose, cod liver oil, sunflower oil, carboxymethyl cellulose, vitamin and mineral mixture (Agrimin) and BHT.

3.8. Feeding

Feeding was done at 1% of the body weight throughout the experiment. The daily ration was divided into only one part and fed at 10.00 am in the morning. The fish were not fed for two days prior to drug administration.

3.9. Chemicals equipment's and laboratory wares

Chemicals of various companies viz. Sigma, Advent chem-bio, Hi-media, Qualichem, and Merck etc. were used. The glassware's used throughout the experiment were a neutral glass of Borosil make and plastic wares were of Tarsons India. Equipment's viz. analytical balance (Shimadzu), a vacuum pump (Millipore), pH meter (Eutech, Thermo scientific, USA), spectrophotometer (Shimadzu), homogenizer, centrifuge (Remi India and Thermo Fischer) and Incubator were used.

3.10. Instrumentation and chromatographic condition

3.10.1. Apparatus

A Waters HPLC system was used in this study the system consists of binary HPLC pump (water 1525), automatic sampler (Waters 2707). The analysis was performed on C-18 reversed-phase (Supelco, Sigma) analytical column (250mm x 4.6 mm, 5.0 µm). The samples were measured at 244 nm using UV-Visible detector (Waters 2489) and data were analyzed with Water Breeze TM 2 integrator system.

Table. 2. HPLC conditions.

Stationary phase	C-18 reversed phase waters bridge column (250 mm×4.6 mm, 5.0µm)
Mobile phase composition	0.1% H_3PO_4 + ACN (60:40)
Elution method	Isocratic
Detector	UV-visible
Wavelength	244 nm
Flow rate	1 ml min^{-1}
Sample temperature	22^0C
Injection volume	10 µl
Runtime	8 min

3.10.2. Standards

Standard of emamectin benzoate was of PESTANAL^R analytical HPLC grade and purchased from Sigma-Aldrich.

3.10.3. Solvents and other reagents

Methanol, Acetonitrile, and water were of HPLC grade and purchased from Advent chem-bio and Merck, Syringe filter (0.22 µm Merck) used for the filtration of sample procured from Merck.

3.10.4. Quantitative assay of EMB

The quantitative assay of EMB was carried out by following the method of Roy *et al.,* (2006) with slight modifications. A mobile phase of 0.1% H_3PO_4: ACN (60:40) was employed on a C-18 reversed phase Sigma SUPELCO analytical column (250 mm ×4.6 mm, 5.0µm) and samples were measured at 244 nm with the UV-visible detector. HPLC system operated isocratically at a flow rate of 1 ml min^{-1}. Elution was performed at a sample temperature of 22^0C and the injection volume was 10 µl. The mobile phase was passed through a 0.20 µm nylon 47 mm filter (Merck) before use in HPLC system.

3.10.5. Stock and standard solution preparation

Stock solution (100 µg ml^{-1}) of EMB was prepared in HPLC grade methanol by dissolving 10 mg of EMB with HPLC grade methanol in 100 ml volumetric flask and made up the volume with methanol. Working Standards of different concentration were prepared by diluting the stock solution with methanol. Working standard solutions were in the range of 100 µg ml^{-1} to 0.05 µg ml^{-1} for EMB.

3.10.6. Calibration curves and linearity

The linearity of the detector response for the compounds was evaluated by injecting a total of 13 working standards solution in duplicates of various concentrations covering the working range of the assay 100 µg ml^{-1} to 0.05 µg ml^{-1}.

3.10.7. Recovery

Recovery test was performed in triplicates by mixing EMB at the concentrations of 0 (as blank), 2, 0.5, 0.2 and 0.1 µg ml^{-1} in the plasma. Recoveries were calculated by comparing the peak area with the respective working standard. The chromatographic conditions are same as mentioned above.

3.11. Preparation of dose of EMB

EMB suspension was made by dissolving in 0.5% CMC (carboxy methyl cellulose). The concentration of EMB suspension was 10 mg ml^{-1} of 0.5% CMC solution. For proper dissolution of EMB, the suspension was stirred overnight.

3.12. Oral administration of EMB suspension

Fifty-four fishes were given a dose of EMB 10 mg kg^{-1} BW, 54 fishes were given a dose of 20 mg kg^{-1} BW and 54 fishes were given a dose of 50 mg kg^{-1} BW. Before oral administration, fish were anesthetized with clove oil (50 µl L^{-1}) and EMB suspension was given to each fish by oral intubation method. Fishes were not fed for one day prior to EMB administration.

3.13. Sample collection

From the tanks which were given a dose of EMB, each fish was anesthetized with clove oil (50 µl L^{-1}) before drawing blood from fish. Blood was drawn from the caudal vein by using a medical syringe using 2.7% EDTA solution. Blood collected was then transferred immediately to an EDTA 2.7% coated eppendorf tube. The tubes containing blood were kept on ice after collection. Blood samples were collected at 0.5, 1, 2, 4, 8, 12, 24, 30, 48, 72, 96,120,144, and 168 h post drug administration. At each time point, blood was collected from three fishes.

3.14. Separation of plasma from blood

Collected blood processed within one hour of blood draw. For separation of plasma collected blood samples centrifuged at 2000 rpm, 4^0C for 20 minutes. After centrifugation supernatant was collected and frozen immediately at -20^0C until further analysis.

3.15. Sample extraction and cleanup

One ml of methanol was added to aliquots of plasma (200 µl) to precipitate protein. After mixing for 5 minutes in the vortex, the sample was centrifuged at 3000g

for 10 minutes and filtered through a nylon filter (0.45 µm). This method was similar to the method used by Garcia *et al.,* (1999).

3.16. HPLC analysis

In order to quantify EMB concentration in plasma at the different time point, 10 µl aliquots of the filtered fractions were injected into HPLC system by auto sampler under the chromatographic conditions described earlier.

3.17. The composition of control feed

A purified diet of crude protein 32.09% and lipid 6% were prepared by using purified ingredients such as casein, gelatin, dextrin, starch soluble, cellulose, cod liver oil, sunflower oil, carboxymethyl cellulose, vitamin and mineral mixture (Agrimin) and BHT.

Table. 3. The composition of the control and medicated feed

Ingredients	**Control feed (g/100g)**	**Medicated feed (g/100g)**
Casein	22.11	22.11
Gelatin	14	14
Dextrin	24	24
Starch soluble	22	21.8
EMB	0	0.2
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**

3.18. Proximate analysis of the control feed

3.18.1. Moisture

The moisture content of the diets and animal tissue were determined by taking a known weight of the sample in the petri dish and drying it in a hot air oven at 100-105°C till a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated by using the following formula.

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample (g)} - \text{Dried weight of sample (g)}}{\text{Wet weight of the sample (g)}} \times 100$$

3.18.2. Crude protein (CP)

The nitrogen content of the sample was estimated quantitatively by Kjeltec semi-automated method (2200 Kjeltec Auto Distillation, Foss Tecator, Sweden) using titration as the means for determining nitrogen percentage. The crude protein percentage was obtained by multiplying the nitrogen percentage by a factor of 6.25.

$$\text{Crude Protein (\%)} = N_2 \text{ (\%)} \times 6.25$$

3.18.3. Ether extracts (EE)

The ether extract was estimated by Soxhlet apparatus using petroleum ether (Boiling point 40-60°C) as the solvent. The calculation was made as follows.

$$\text{Ether extract (\%)} = \frac{\text{Weight of the ether extract (g)}}{\text{Weight of the sample (g)}} \times 100$$

3.18.4. Ash

Ash content was estimated by taking a known weight of the sample in a silica crucible and placing it in a muffle furnace at 600°C for 6 hours. The calculation was done as follows

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

3.18.5. Total carbohydrate (TC)

The total carbohydrate (TC) of the tissue was calculated by subtracting the percentage of other nutrients from 100 (Hasting, 1969)

TC (%) =100 – Crude protein (%) – Ether extract (%) – Ash (%)

However, NFE was calculated as, NFE= 100 – (CP % + EE% + Ash%+ CF %)

3.19. Physico-chemical parameters of water

Water quality parameters viz. Temperature, pH dissolved oxygen, free carbon dioxide, total hardness„ ammonia, nitrite, and nitrate were recorded during the experimental period.

3.19.1. Temperature

The water temperature of all the experimental tubs was recorded using multiparameter water quality recorder (MERCK Germany) in which a temperature probe was also provided.

3.19.2. pH

The pH was measured by a digital pH meter (Eutech, USA) in the experimental tanks.

3.19.3. Dissolved oxygen

The dissolved oxygen was measured by membrane electrode method using dissolved oxygen meter (MERCK, Germany) for all the experimental tubs.

3.19.4 Free carbon dioxide

The dissolved free carbon dioxide was measured by the titrimetric method (APHA, 1998) and calculated using the following formula,

$$CO_2 \text{ (mg } L^{-1}) = \frac{A \times N \times 44 \times 1000}{\text{Volume of the sample (ml)}}$$

Where, A = Volume of titrant (NaOH)

N = Normality of titrant (N/44)

3.19.5. Carbonate hardness

Carbonate hardness was estimated by carbonate hardness test kit (Carbonate hardness test, MERCK, Germany).

3.19.6. Ammonia

Un-ionized ammonia concentration was estimated spectrophotometrically at 635 nm wavelength by phenate method (APHA, 1998) and compared with the standard graph. The concentration was expressed as mg L^{-1}.

3.19.7. Nitrite-N

Nitrite concentration was estimated spectrophotometrically at the 543 nm wavelength (APHA, 1998) and compared with the standard graph. The concentration was expressed as mg L^{-1}.

3.19.8. Nitrate-N

Nitrate concentration was estimated spectrophotometrically at 543 nm wavelength (APHA, 1998) and compared with the standard graph. The concentration is expressed as mg L^{-1}.

3.20. Enzyme assays

3.20.1. Tissue homogenate preparation

The muscle, liver, gill, and intestine of fishes were removed carefully and were weighed. It was homogenized with chilled sucrose solution (0.25 M) in a glass tube using tissue homogenizer. The tube was continuously kept on ice to avoid heating. The homogenate was centrifuged at 5000 rpm for 10 minutes at 4ºC in a cooling centrifuge machine. The supernatant was stored at 4ºC until the use. A 5% homogenate was prepared for muscle, liver, gill, and intestine.

3.20.2. Protein estimation

Total protein of each tissue sample was estimated by Lowry method (Lowry, 1951). Aliquots (20 µL) of the sample were taken in dry test tubes and volume raised to 2 ml by adding alkaline copper sulfate reagent. After mixing, the tubes were kept for incubation at room temperature for 10 min. To these tubes, 0.2 ml folin ciocalteau solution was added. The content was mixed on a cyclomixer and allowed to incubate for 30 min. The absorbance was taken at 660 nm and plotted on the standard curve to obtain the total protein content of the tissue sample. The standard curve was made using bovine serum albumin (BSA) as the standard.

3.20.3. Enzymes of oxidative stress

3.20.3.1. Superoxide dismutase (SOD)

Superoxide dismutase was assayed according to the method described by Misra and Fridovich (1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme. Fifty microliters of the sample were taken in the cuvette and

1.5 ml 0.1 M carbonate-bicarbonate buffer containing 57 mg dl^{-1} EDTA (pH 10.2) and 0.5 ml epinephrine (3 mM) was added and mixed well. Change in optical density at 480 nm was read continuously for 3 min in a Shimadzu - UV spectrophotometer. One unit of SOD activity was the amount of protein required to give 50% inhibition of epinephrine auto-oxidation. SOD is expressed as units per mg protein.

3.20.3.2. Catalase (CAT)

Catalase was assayed according to the method described by Takahara *et al.,* (1960). To a reaction mixture of 245 ml phosphate buffer (50 mM, pH 7.0), enzyme source was added and the reaction was started by the addition of 1.0 ml of H_2O_2 solution. The decrease in the absorbance was measured at 240 nm at 30s intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of the H_2O_2 solution. Enzyme activity was expressed in nanomoles of H_2O_2 decomposed min^{-1} mg $protein^{-1}$.

3.20.3.3. Glutathione S-transferase (GST)

Glutathione S-Transferase (GST) catalyzes the conjugation of glutathione to numerous potentially toxic compounds, including aliphatic, aromatic, heterocyclic radicals, epoxides, and arene oxides GST activity can be determined by the method of Habig *et al.,* (1974). Aliquots of 0.1 ml tissue homogenate, 1 ml, 0.3 M phosphate buffer, 0.1 ml 30 mM 1-chloro-2-4 dinitrobenzene (CDNB) in methanol were pipetted into a cuvette. The mixture was made to 2.7 ml by adding 1.5 ml of distilled water and incubated at 37^0C for 5 min. after setting the spectrophotometer at 340 nm, 0.1 ml of 30 mM reduced glutathione (GSH) was pipetted into the mixture and change in absorbance for 3 min at 15 sec intervals was recorded for each sample in triplicates. Distilled water was used as a blank. Enzyme activity was expressed as nano-moles of CDNB conjugated formed per minute per milligram protein or specific activity is expressed per mg of protein.

3.20.3.4. Lactate dehydrogenase (LDH)

LDH catalyzes the conversion of lactate to pyruvic acid and back, which is a key metabolic step in glycolysis and other metabolic pathways (Everse & Kaplan, 2012). A dehydrogenase is an enzyme that transfers a hydride from one molecule to another. Tissue homogenate was mixed with sodium pyruvate in the presence of NADH and the rate of the NADH to NAD was measured by the reduction of the optical density of the mixture at 340 nm. The LDH activity was assayed by the method of Wroblewski and Laduce (1955). The total 3 ml of a reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of tissue homogenate, 0.1 ml of NADH solution (2 mg NADH dissolved in 1 ml of PO_4 buffer solution) and 0.1 ml sodium pyruvate. The reaction was started after addition of substrate sodium pyruvate. The OD was recorded at 340 nm at 30 sec intervals. The enzymatic activity was expressed as unit's mg protein^{-1} min^{-1} at 25^{0}C where 1 unit equal was to Δ0.01 OD min^{-1}.

3.20.3.5. Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) catalyzes the reduction of hydroperoxide, including hydrogen peroxides, by reduced glutathione and functions to protect the cell from oxidative damage. Oxidized glutathione (GSSG), produced upon reduction of an organic hydroperoxide by GPx and is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. The rate of decrease in the A340 is directly proportional to the GPx activity in the sample. GPx activity was estimated according to the method described by Flohe and Gunzler (1984). According to the method perfused tissue were homogenized (10% w/v) in 50 mM phosphate buffer, pH 7.0, containing 1 mM EDTA, 1 mM PMSF and 250 mM sucrose. The cytosolic fraction was used as the enzyme source to estimate the peroxide activity. The activity was measured by monitoring the oxidation of NADPH at 340 nm in a reaction mixture containing 50 µl of the enzyme, 250 mM phosphate buffer, pH 7.0, 2.5 mM EDTA, 2.5 mM sodium azide, 1 mM GSH, 2 mM NADPH, one unit of GR incubated for five minutes at room temperature. Total GSH Px activity was assayed by using 1.5 mM cumene hydroperoxide. The Se dependent GSH Px activity was assayed

by using 12 mM H_2O_2. Specific activity is expressed as units per mg protein, where one unit defined as one nmole of NADPH oxidized per min.

3.20.3.6. Aspartate aminotransferase (Serum GOT)

Serum GOT was done by Erba Mannheim kit following the manufacturer's instructions. The reaction mixture contained 500 µl working reagent and 25 µl serum sample. After mixing the reaction mixture properly absorbance was measured at 340 and 370 nm at kinetic mode.

3.20.3.7. Alanine aminotransferase (Serum GPT)

Serum GPT was done by Erba Mannheim kit following the manufacturer's instructions. The reaction mixture contained 500 µl working reagent and 25 µl serum sample. After mixing the reaction mixture properly absorbance was measured at 340 and 370 nm at kinetic mode.

3.20.3.8. Alkaline phosphatase (ALP)

ALP activity was determined by the method of Garen and Levinthal (1960). The assay mixture comprised of 0.2 ml bicarbonate buffer (0.2 M), 0.1 ml of 1 M $MgCl_2$, 0.1 ml tissue homogenate, 0.5 ml distilled water and 0.1 ml of freshly prepared 0.1 M para-nitrophenyl phosphate. The reaction mixture was incubated at 37^0C for 15 min and the reaction was stopped by 1 ml of NaOH and OD was taken at 410 nm.

3.21. Bioaccumulation analysis of EMB from muscle

The residue of the EMB in muscle was analyzed by homogenizing 1 g muscle in 5 ml of 0.9% NaCl solution followed by centrifugation (5000 rpm for 10 min), sonication of supernatant and centrifuge after sonication. Later 250 µl of supernatant mixed with 1ml of methanol followed by centrifugation (3000g for 10 min), filtering through a 0.45 µ syringe filter into the vial and 10 µl injected to HPLC.

3.22. EROD analysis

3.22.1. Chemical reagents and kits

Chemicals such as EDTA, 2-amino-2-(hydroxymethyl) propane-1-3-diol (TRIS), ammonium acetate, glacial acetic acid, glycerol, acetylacetone, potassium chloride, formaldehyde and sulphuric acid (98%) were procured from Merck, India. Ethoxyresorufin, NADPH, sodium hydrosulfite, 34 technical grades (85%) was provided by Sigma Aldrich, USA, and resorufin (cyman). All chemicals were of analytical grade.

3.22.2. Experimental animal and design of the experiment

Twenty-one fishes of *Labeo rohita*, weighing 90±10 g were kept placed three tanks each tank contains 7 fishes. The dose of EMB was @ 20 mg kg^{-1} of fish. The fishes were then sacrificed at the end of 3, 6, 9, 12 and 15 days and hepatic microsome was isolated.

3.22.3. Microsome isolation

Microsome was obtained from liver following the method prescribed by Nilsen *et al.*, (1998). Liver tissues were thawed and homogenized (approximately 1 g liver tissue) in four volumes of homogenization buffer (0.08 M Na_2HPO_4; 0.02 M KH_2PO_4; 0.15 M KCl, pH 7.4). Homogenate was centrifuged at 10.000 x g for 20 min at 4ºC. The supernatant was ultracentrifuge (BECKMAN, Coulter, U.S.A) at 100.000 x g for 60 min at 4°C. The pellet was resuspended in 1ml of resuspension buffer (homogenization buffer with 20% glycerol, v/v) to obtain the hepatic microsomal fraction. Protein concentration was determined by using bovine serum albumin (BSA) as a standard Bradford (1976). All the above steps were carried out at 4ºC.

3. 22.4. EROD analysis of CYP1A.

EROD analysis was performed using a method described by Nilsen *et al.,* (1998). Samples of microsome isolation, freshly prepared or thawed from -80°C for the first time, on ice until analysis, the assay was performed controlled cuvettes at room

temperature. Set the excitation and emission monochromators of the spectrofluorometry (RF-5301 PC, Shimadzu) to 535 nm and 585 nm, respectively NADPH (0.1 M) was added to a reaction mixture containing microsomal protein (10 µl), 7-ethoxyresorufin (0.097 mg/ml in methanol). Measured resorufin production over 2-3 min by recording the change in fluorescence. 10 µl, resorufin solution to the cuvette as an internal standard. Calculate the specific activity of the enzyme (pmol min^{-1}mg $protein^{-1}$) in the sample by using the following formula;

pmoles resorufin/min/mg protein= Fs/min×R/FR×1/Vs×1/Cs

Fs/min = Increase in sample fluorescence per min,

R = Amount of resorufin added as internal standard (pmol),

FR = Increase in fluorescence owing to resorufin standard,

Vs = Sample volume (ml),

Cs = Protein concentration of sample (mg mL^{-1})

3.23. Statistical analysis

The data were statistically analyzed by using statistical package SPSS version 16 in which data were subjected to one-way ANOVA and Duncan's multiple range tests was used to determine the significant differences between the means.

4. RESULTS

4.1. Physico-chemical parameters of water

The physiochemical parameters of water such as temperature (^{0}C), pH, oxygen (mg L^{-1}), free carbon dioxide (mg L^{-1}), total hardness (mg L^{-1}), ammonia (mg L^{-1}), Nitrite-N (mg L^{-1}), Nitrate-n (mg L^{-1}), were recorded during the experimental period an average of all the treatments are presented in table 4.

4.1.1. Temperature

The water temperature of the different experimental group was ranged from 28^{0}C to 30^{0}C throughout the experimental period.

4.1.2. pH

There was no much variation in the pH values during the experimental period. The pH values were recorded within the range of 7.8 to 8.1.

4.1.3. Dissolved oxygen

The dissolved oxygen concentrations of all the experimental tanks were recorded within the range of 6.7 to 7.1 mg L^{-1} during the experimental period.

4.1.4. Free carbon dioxide

The free carbon dioxide in water was found to be negligible during the experimental period.

4.1.5. Carbonate hardness

The carbonate hardness was found to be 238 - 245 mg L^{-1} during the experimental period.

4.1.6. Total ammonia - N

The total ammonia content of all the experimental tanks was recorded before water exchange. It was found to be in the range of 0.05 to 0.1 mg L^{-1}.

4.1.7. Nitrite – N

The nitrite - N content was found to be in the range of 0.05 to 0.1 mg L^{-1}.

4.1.8. Nitrate — N

The nitrate- N content was found to be in the range of 0.5 to 0.1 mg L^{-1}, throughout the experimental period.

Table. 4. Physico-chemical parameters of water during the experimental period of 15 days for different experimental groups.

	Temperature (^{0}C)	pH	DO (mg L^{-1})	Free CO_2	Hardness (mg L^{-1})	Ammonia (mg L^{-1})	Nitrite (mg L^{-1})	Nitrate (mg L^{-1})
0 day	29-30	7.9-8.0	6.8-7.1	ND	238-240	0.05-0.1	0.07-0.09	0.5-0.9
3 day	28-29	7.8-7.9	6.7-6.9	ND	241-245	0.06-0.1	0.05-0.08	0.8-1.0
6 day	29-30	7.8-8.1	6.9-7.1	ND	238-242	0.07-0.08	0.07-0.08	0.6-0.9
9 day	28-30	7.9-8.1	6.8-7.0	ND	239-243	0.09-0.1	0.07-0.08	0.7-1.0
12 day	28-30	7.9-8.0	6.9-7.1	ND	239-245	0.08-0.09	0.09-0.10	0.7-0.9
15 day	29-30	7.8-8.0	6.7-7.0	ND	240-242	0.07-0.1	0.06-0.08	0.6-0.7

ND- Not Detected

4.2. Quantification of emamectin benzoate (EMB)

4.2.1. Standard curve of EMB

Standard curves were plotted using EMB at a concentration range of 0.05-100 µg ml^{-1} in triplicate respectively. Regression analysis of standard concentration data

was done by following equation Y = 2.73e+003 X + 9.67e+002 and the linearity of curve were 0.972432 in first order (figure 1).

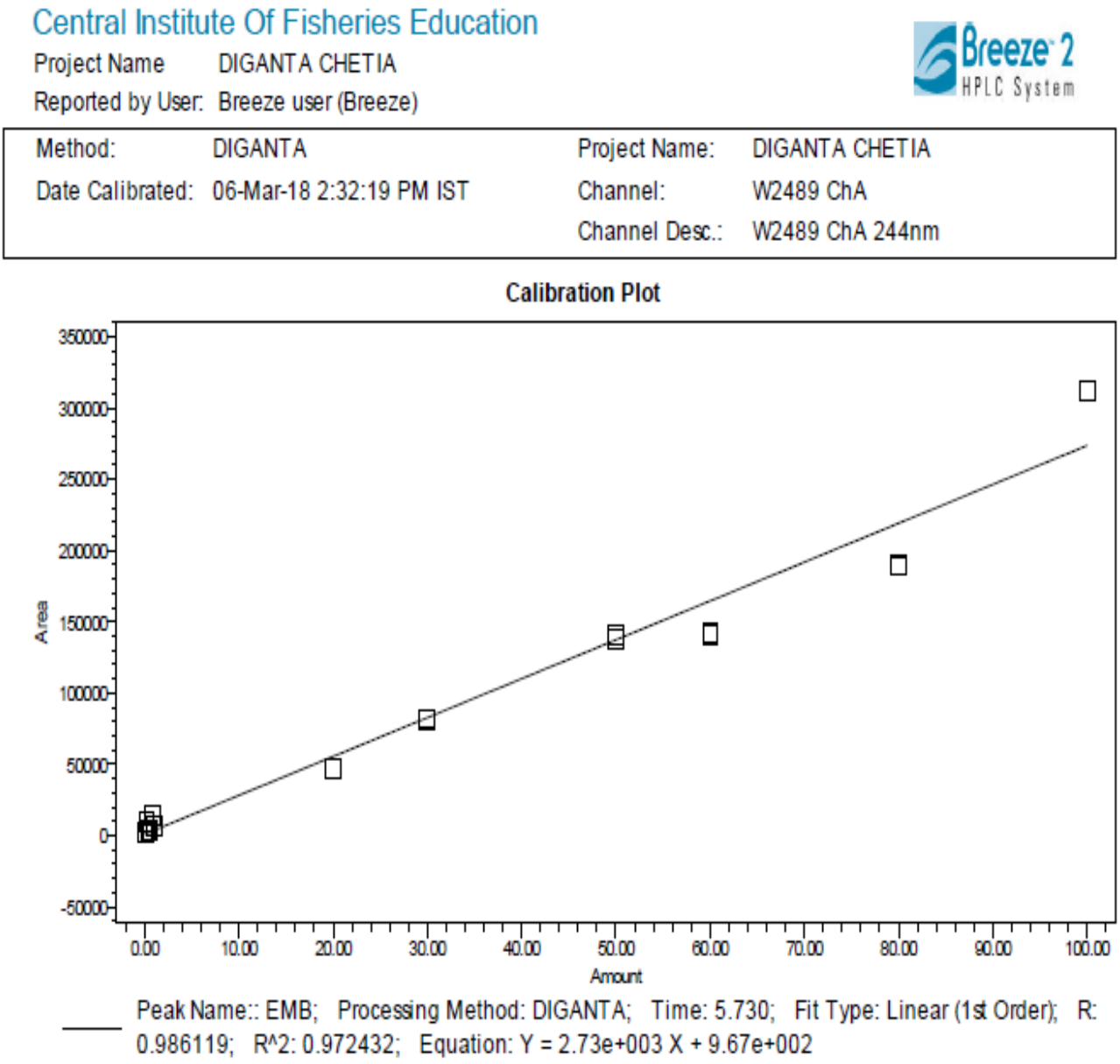


Fig. 1. Calibration curve of EMB using standard concentration in the range of 0.05 to the 100 µg ml^{-1}.

4.2.2. Plasma concentration of EMB

The depletion profile of EMB in plasma was studied at different time intervals after oral intubation of a single dose of EMB @ 10 mg kg^{-1}, 20 mg kg^{-1} and 50 mg kg^{-1} body weight of the *Labeo rohita* and timeline graph were presented in figure 2 and 17 respectively. The parent residue of EMB was not detected in plasma after administration of 10 mg kg^{-1} body weight (BW), whereas at higher doses @ 20 mg kg^{-1} and 50 mg kg^{-1} BW, it was detected in plasma up to 120 h post-administration of @ 20 mg kg^{-1} and up to 144 h of post-administration @ 50 mg kg^{-1} BW respectively. In 20 mg kg^{-1} BW dosage, the plasma concentration of EMB at 0.5 h was 47.04 ± 0.02 µg ml^{-1}, which was gradually increased up to the maximum level of 51.72 ± 0.02 µg ml^{-1} (C_{max}) at 1 h (T_{max}). Afterward, there was a significant ($p < 0.00$) decrease in plasma EMB concentration from 1 h to 120 h (figure 3 to 16). On the other hand, in case of 50 mg kg^{-1} BW dose, the plasma concentration of EMB at 0.5 h was 5.02 ± 0.01 µg ml^{-1} and which was increased up to 78.53 ± 0.01 µg ml^{-1} (C_{max}) at 1 h (T_{max}). Afterward, there was a significant decrease in plasma EMB concentration from 1 h to 144 h (figure 18 to 31). The plasma concentration of EMB after administration of single dose of 20 mg kg^{-1} BW through the feed at 0.5 h was 10.32 ± 0.01 µg ml^{-1}, and which was increased up to 21.82 ± 0.01 µg ml^{-1} (C_{max}) at 1 h (T_{max}). Afterward, there was a significant decrease in plasma EMB concentration up to 96 h (figure 33 to 45).

Table. 5. Mean plasma concentration of EMB after dose @ 20 mg/kg body weight.

Time after drug administration (h)	The concentration of EMB (µg ml^{-1})
0.5	47.04^{b} ± 0.02
1	51.72^{a} ± 0.02
2	39.57^{c} ± 0.00
4	37.55^{d} ± 0.01
8	33.49^{e} ± 0.01

12	$28.29^{f} \pm 0.02$
24	$12.74^{g} \pm 0.07$
36	$7.72^{h} \pm 0.00$
48	$3.85^{i} \pm 0.02$
72	$2.98^{j} \pm 0.00$
96	$2.68^{k} \pm 0.00$
120	$2.42^{l} \pm 0.00$
140	ND

Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

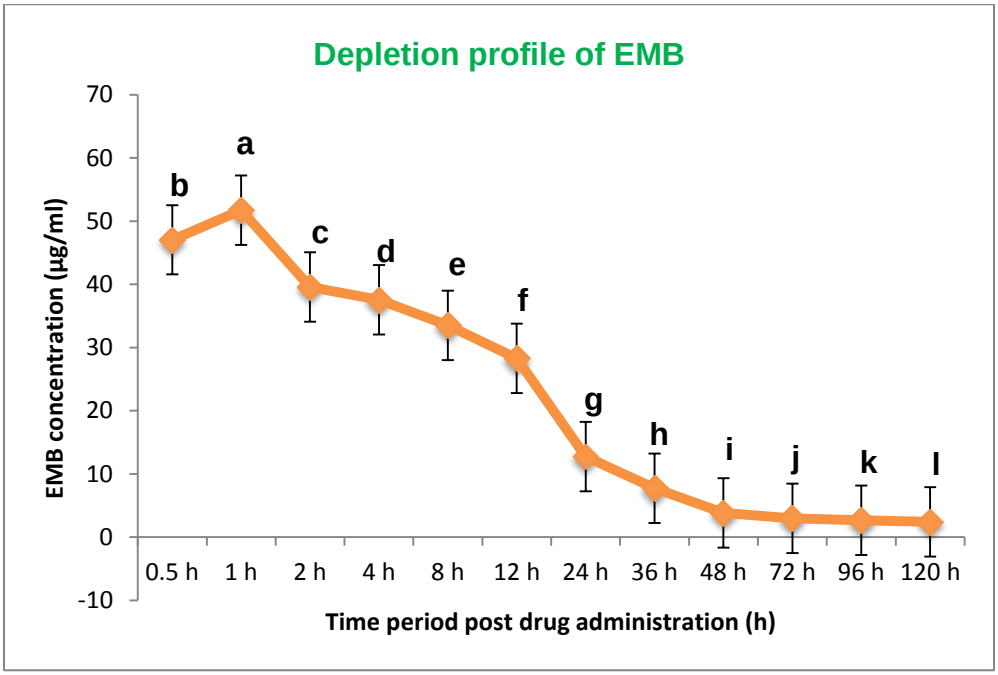


Fig. 2. Depletion profile of plasma EMB concentration after oral administration of @ 20 mg kg^{-1} BW.

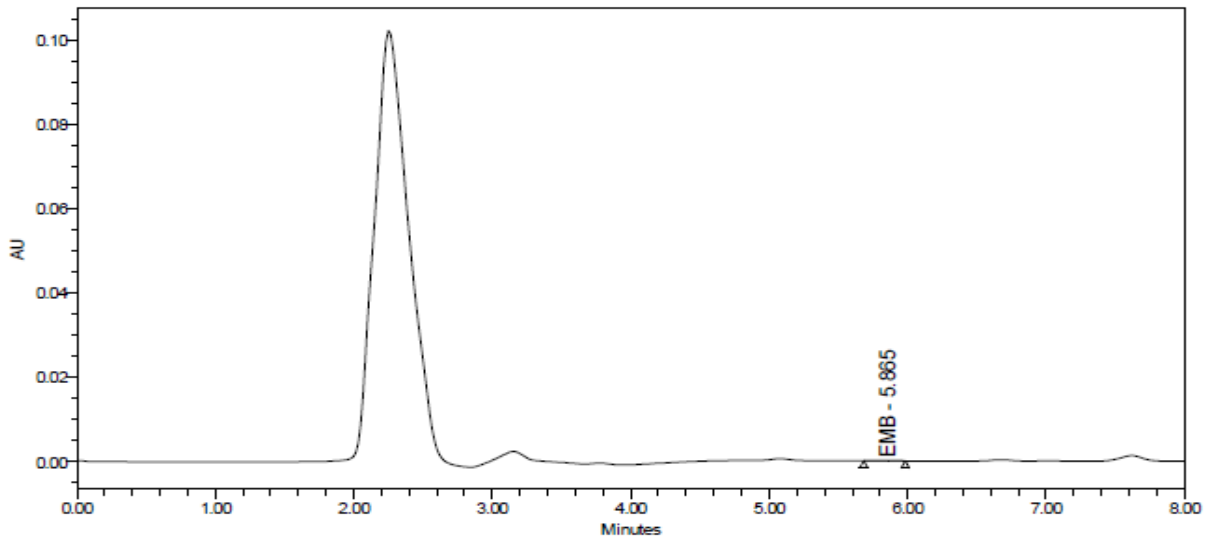
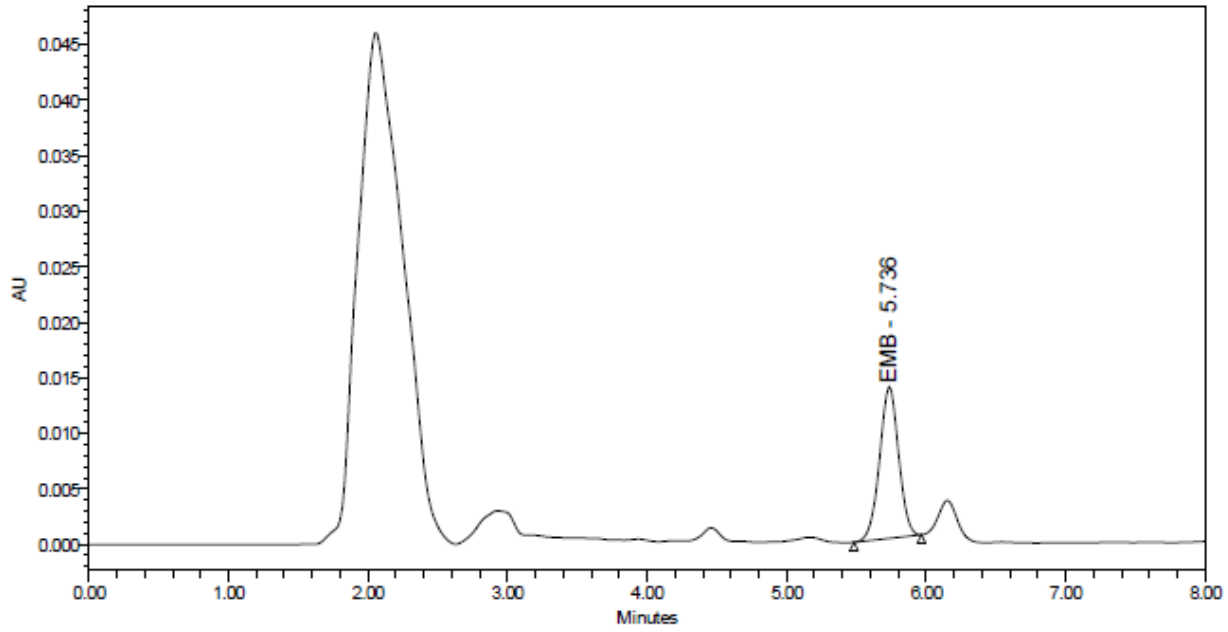
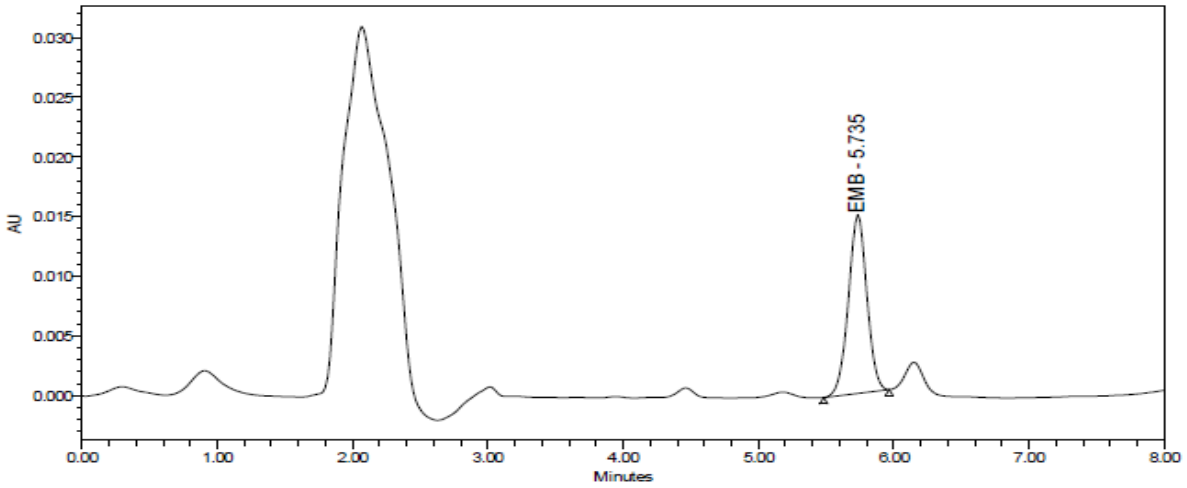


Fig. 3. Chromatogram of control plasma sample



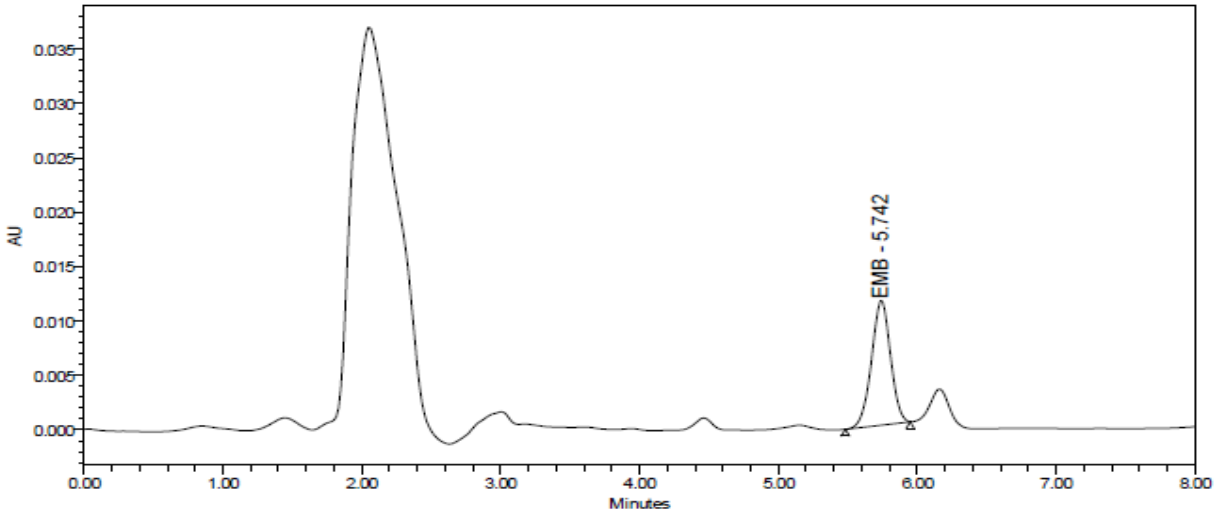
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.736	129286	100.00	13671	100.00	47.064

Fig. 4. Chromatogram of EMB in plasma at 0.5 h post dose of @ 20 mg kg^{-1} BW.



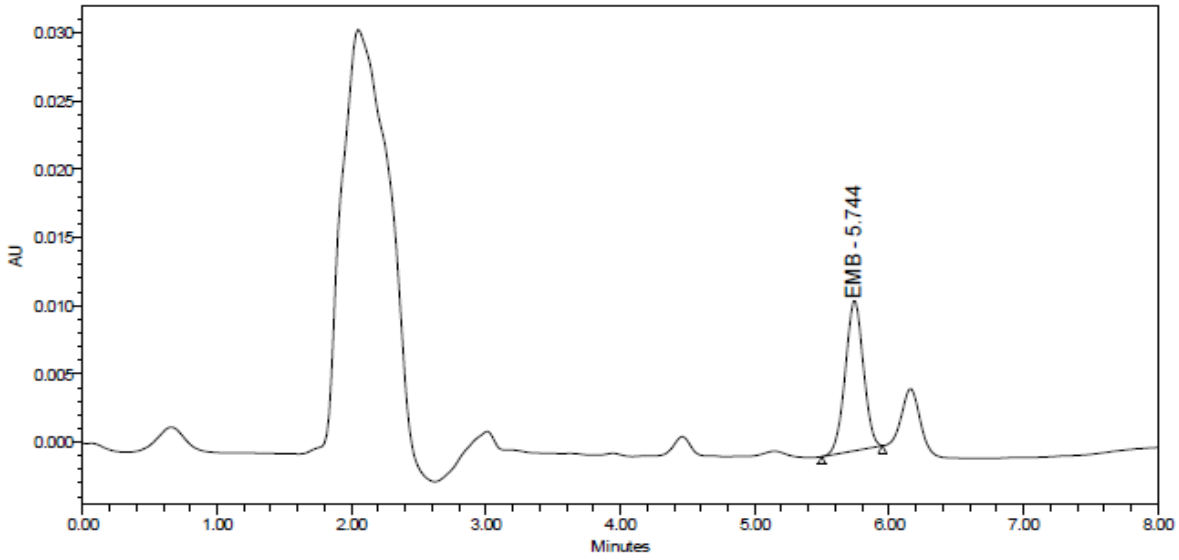
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.735	142047	100.00	14959	100.00	51.744

Fig. 5. Chromatogram of EMB in plasma at 1 h post dose of @ 20 mg kg^{-1} BW.



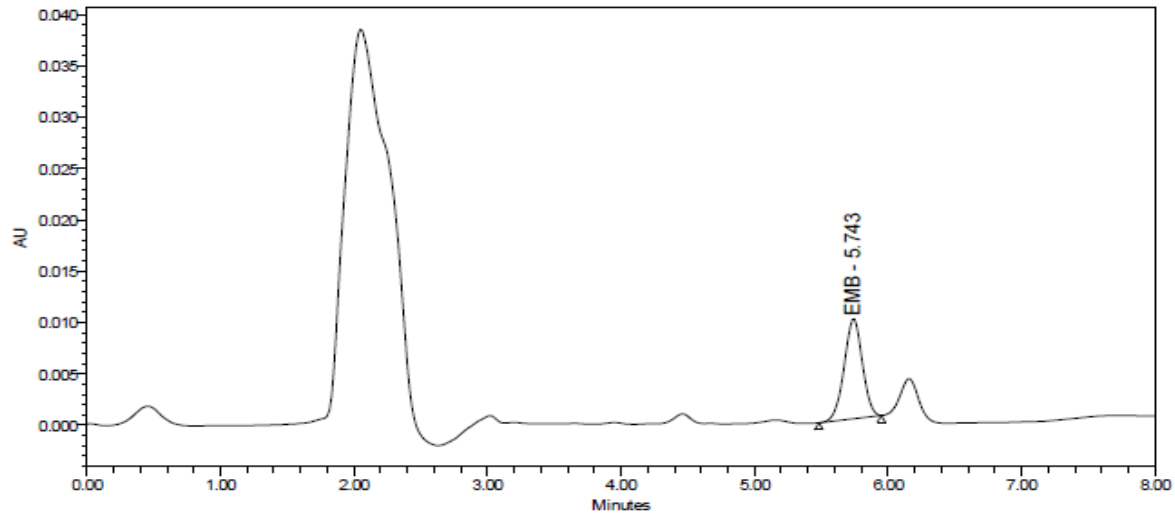
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.742	108856	100.00	11473	100.00	39.571

Fig. 6. Chromatogram of EMB in plasma at 2 h post dose of @ 20 mg kg^{-1} BW.



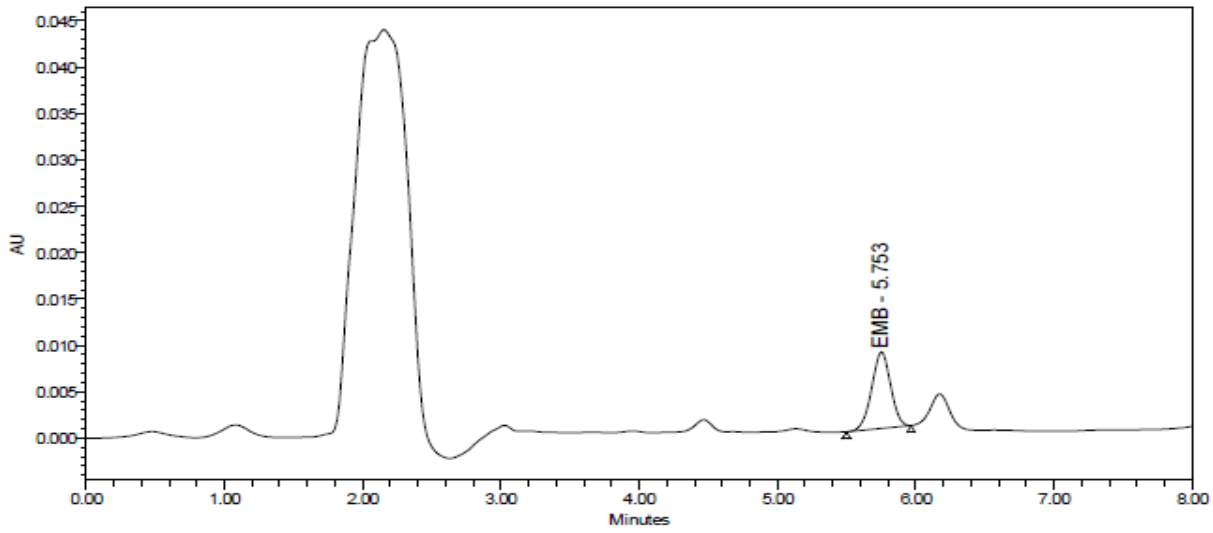
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.744	103293	100.00	11021	100.00	37.530

Fig. 7. Chromatogram of EMB in plasma at 4 h post dose of @ 20 mg kg^{-1} BW.



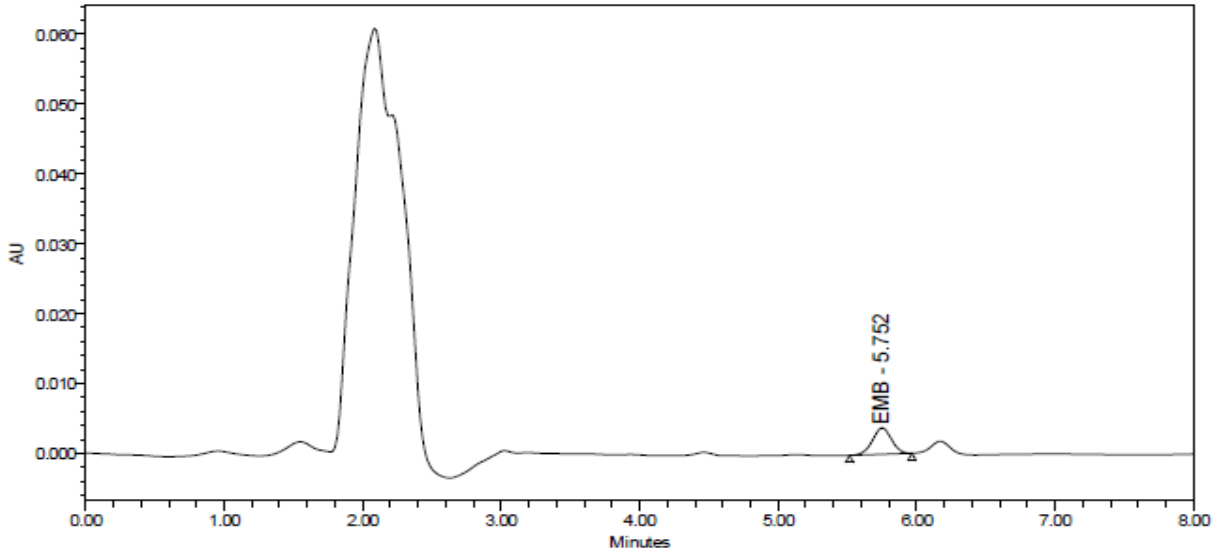
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.743	92344	100.00	9769	100.00	33.515

Fig. 8. Chromatogram of EMB in plasma at 8 h post dose of @ 20 mg kg^{-1} BW.



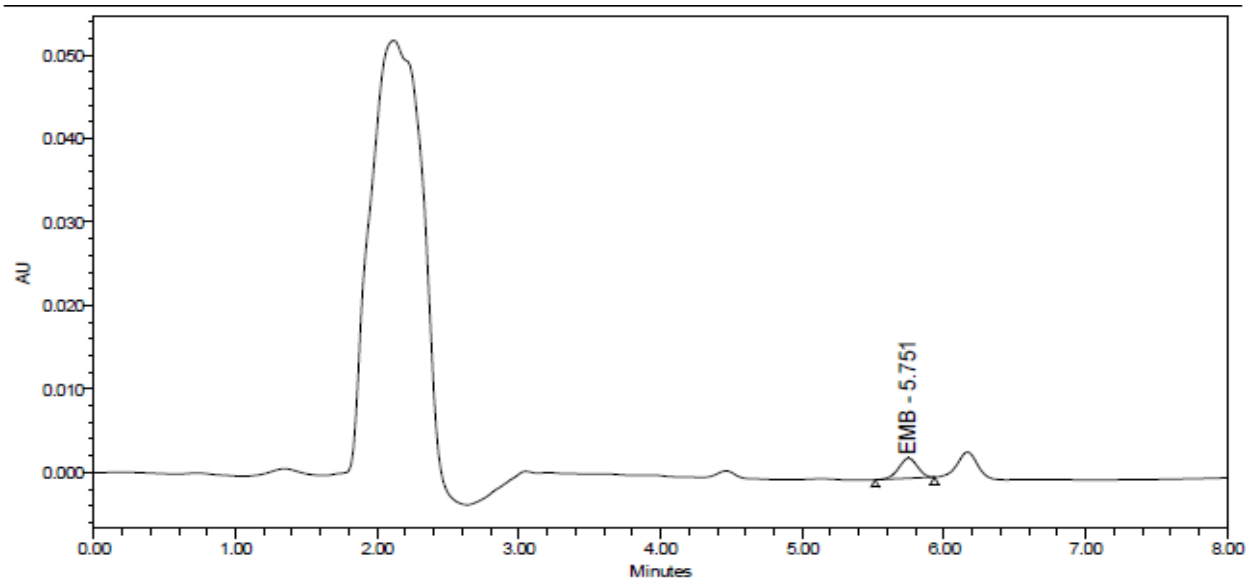
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.753	78055	100.00	8281	100.00	28.274

Fig. 9. Chromatogram of EMB in plasma at 12 h post dose of @ 20 mg kg^{-1} BW.



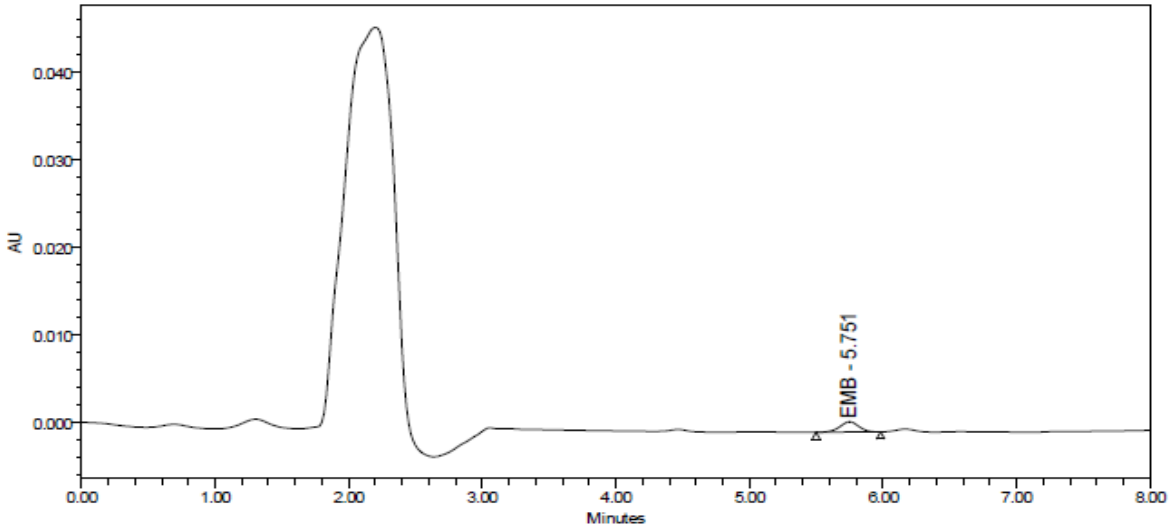
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.752	35906	100.00	3812	100.00	12.815

Fig. 10. Chromatogram of EMB in plasma at 24 h post dose of @ 20 mg kg^{-1} BW.



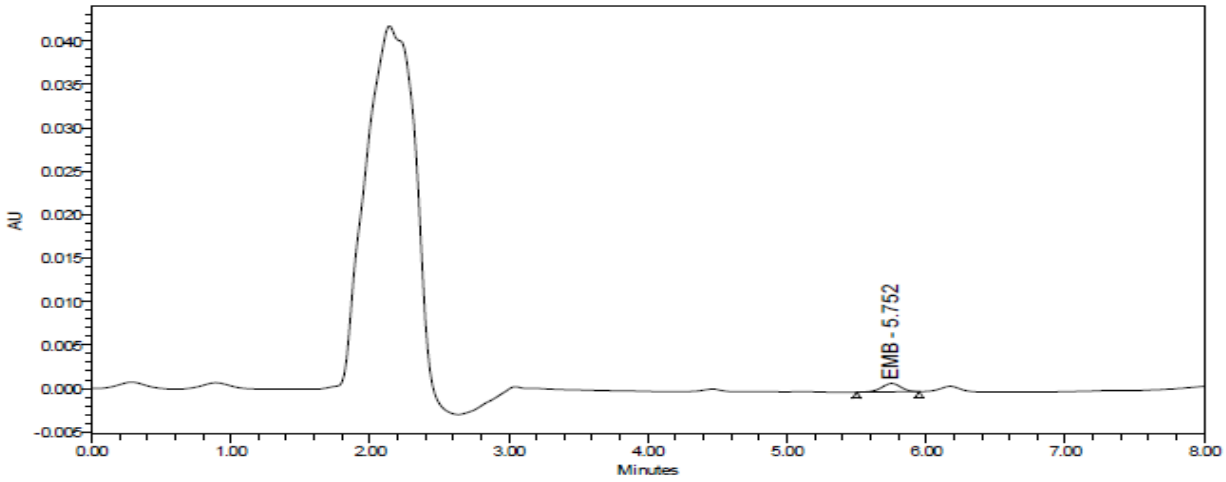
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.751	22052	100.00	2426	100.00	7.734

Fig. 11. Chromatogram of EMB in plasma at 36 h post dose of @ 20 mg kg^{-1} BW.



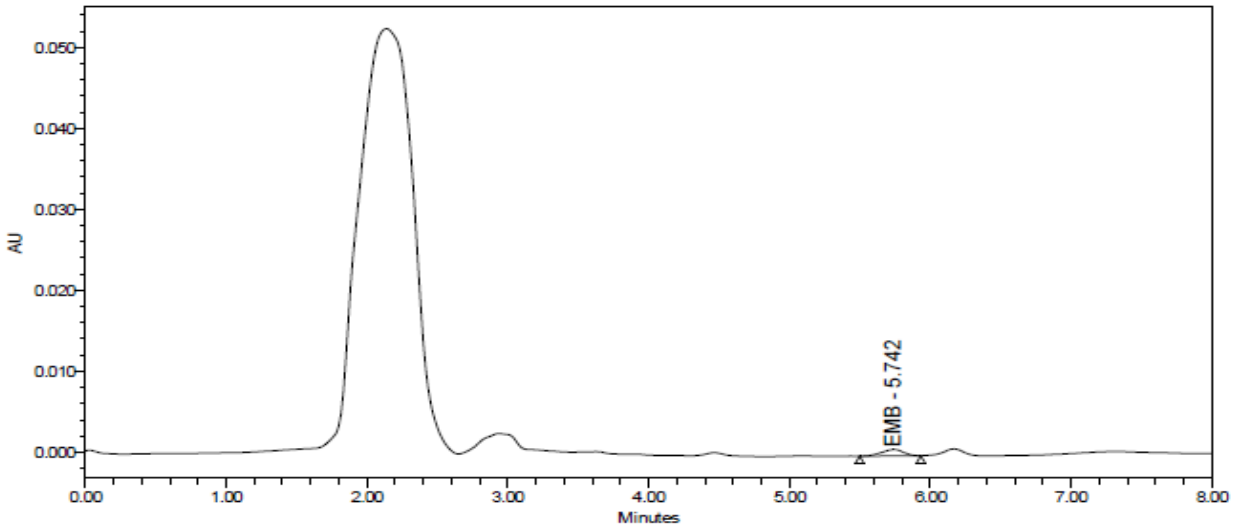
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.751	11508	100.00	1189	100.00	3.866

Fig. 12. Chromatogram of EMB in plasma at 48 h post dose of @ 20 mg kg^{-1} BW.



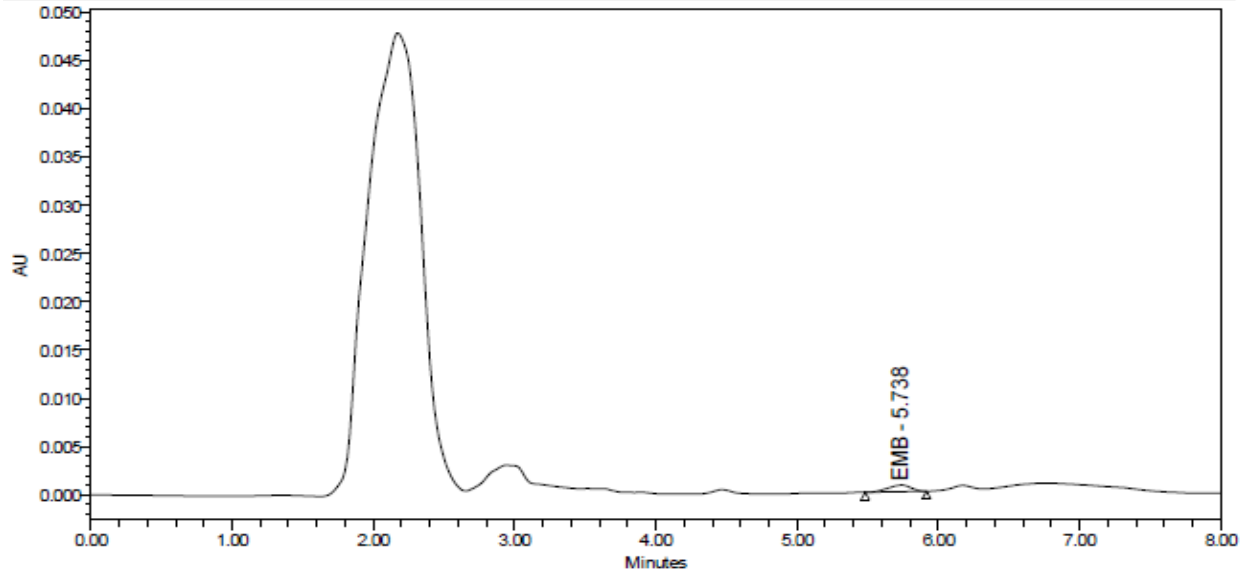
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.752	9117	100.00	963	100.00	2.989

Fig. 13. Chromatogram of EMB in plasma at 72 h post dose of @ 20 mg kg^{-1} BW.



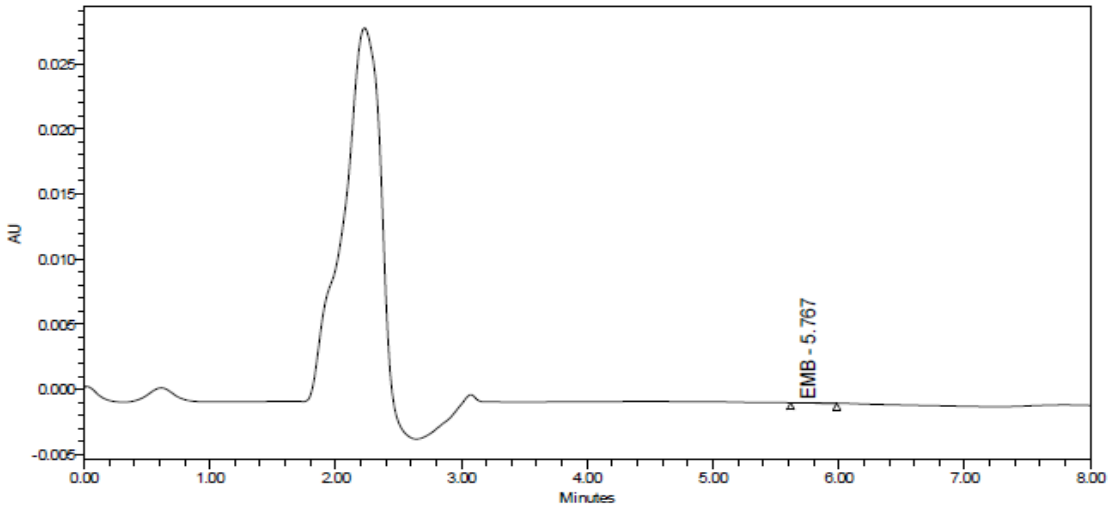
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.742	8317	100.00	796	100.00	2.696

Fig. 14. Chromatogram of EMB in plasma at 96 h post dose of @ 20 mg kg^{-1} BW.



Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.742	7565	100.00	675	100.00	2.420

Fig. 15. Chromatogram of EMB in plasma at 120 h post dose of @ 20 mg kg^{-1} BW.



Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.767	192	100.00	16	100.00	0

Fig. 16. Chromatogram of EMB in plasma at 144 h post dose of @ 20 mg kg^{-1} BW.

Table. 6. EMB concentration in plasma after post administration dose of @ 50 mg kg^{-1} body weight.

Time after drug administration (h)	Plasma concentration of EMB (µg ml^{-1})
0.5	$5.02^{k} \pm 0.01$
1	$78.53^{a} \pm 0.01$
2	$71.29^{b} \pm 0.00$
4	$52.93^{c} \pm 0.04$
8	$37.88^{d} \pm 0.00$
12	$31.15^{e} \pm 0.01$
24	$28.08^{f} \pm 0.00$
36	$24.06^{g} \pm 0.01$
48	$19.58^{h} \pm 0.00$
72	$11.45^{i} \pm 0.02$
96	$6.07^{j} \pm 0.00$
120	$3.68^{l} \pm 0.01$
144	$2.10^{m} \pm 0.00$
168	ND

Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

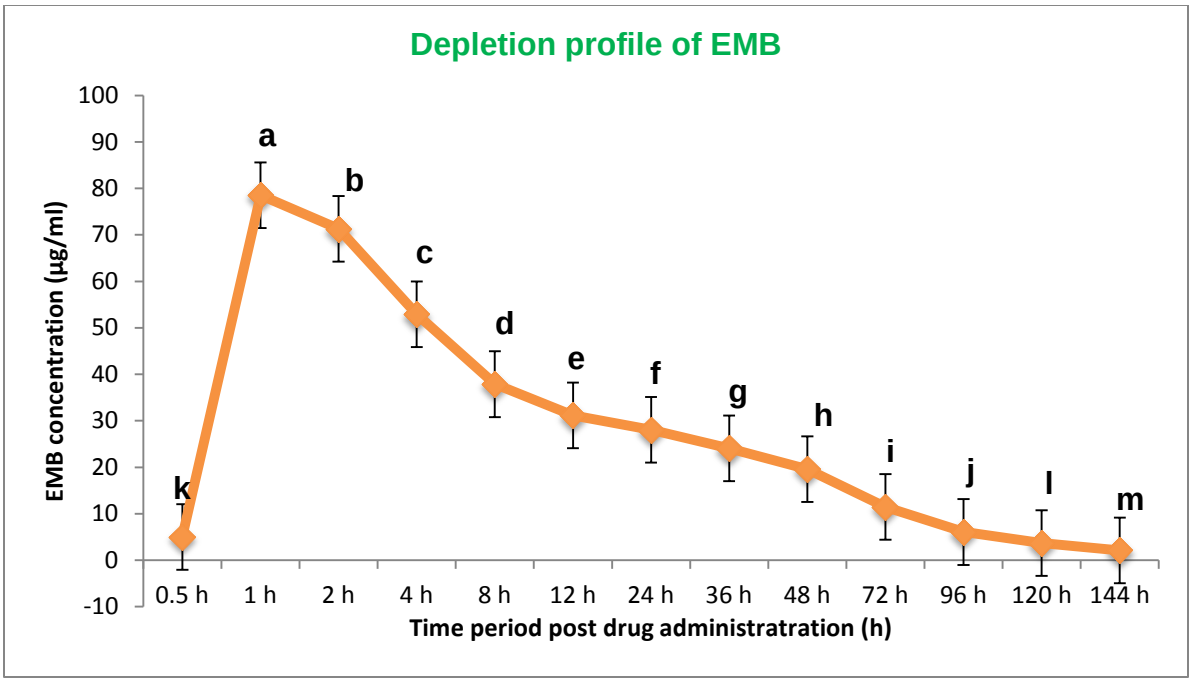
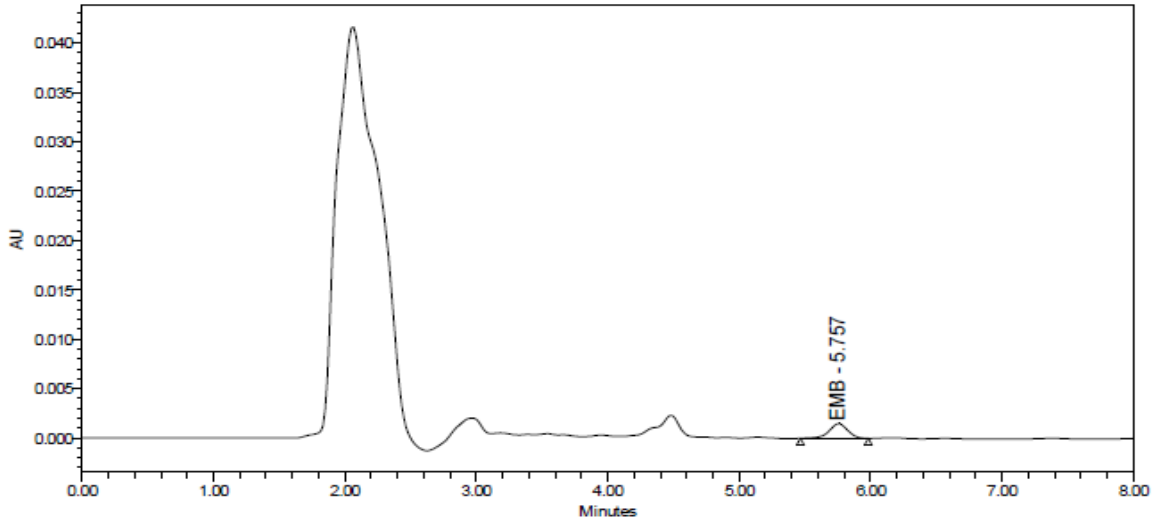
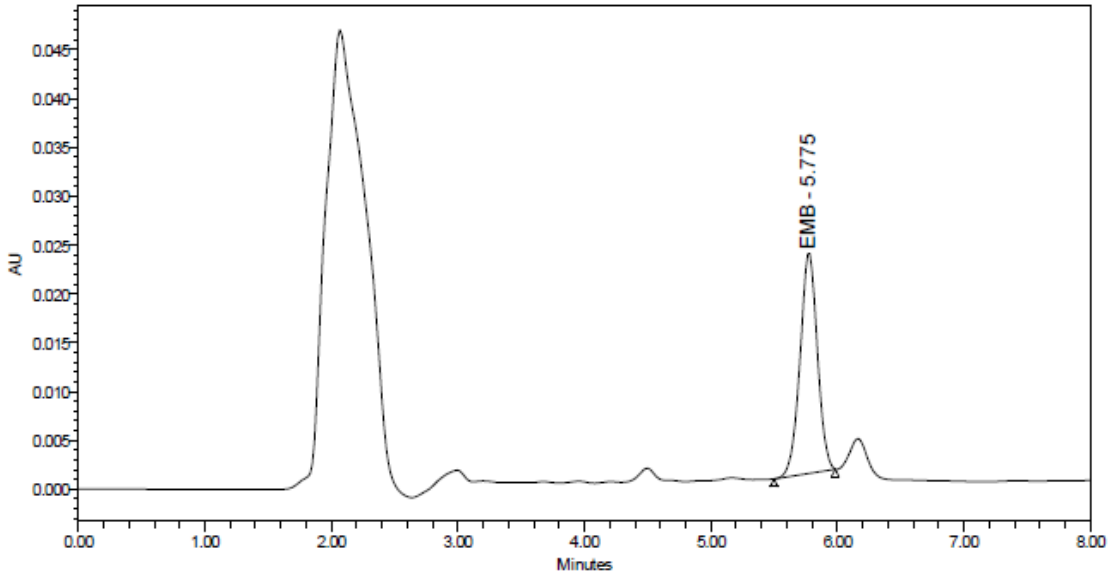


Fig. 17. Depletion profile of plasma EMB concentration after oral administration of @ 50 mg kg^{-1} BW.



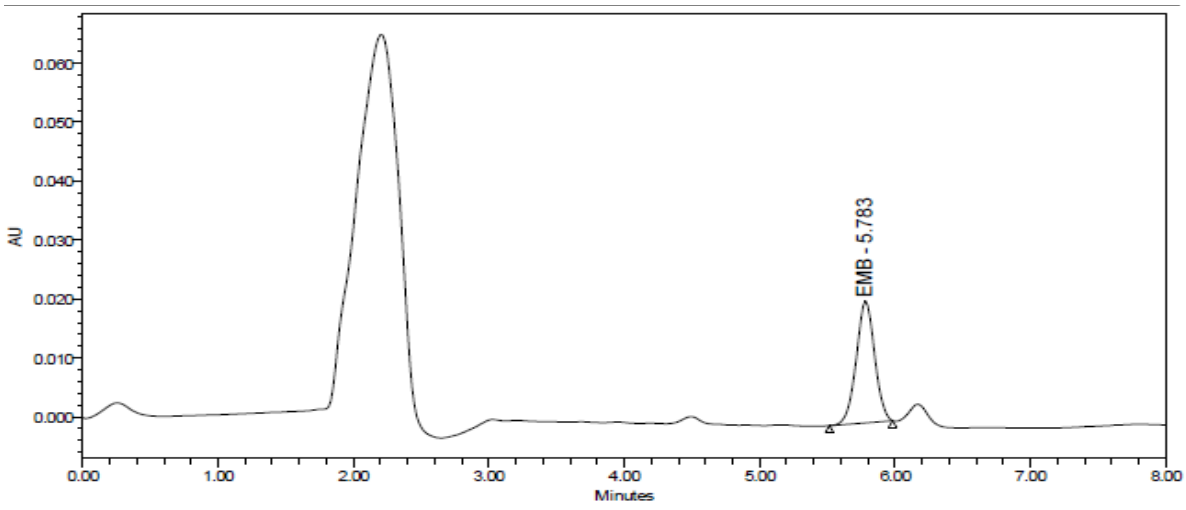
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.757	14680	100.00	1497	100.00	5.030

Fig. 18. Chromatogram of EMB in plasma at 0.5 h post dose of @ 50 mg kg^{-1} BW.



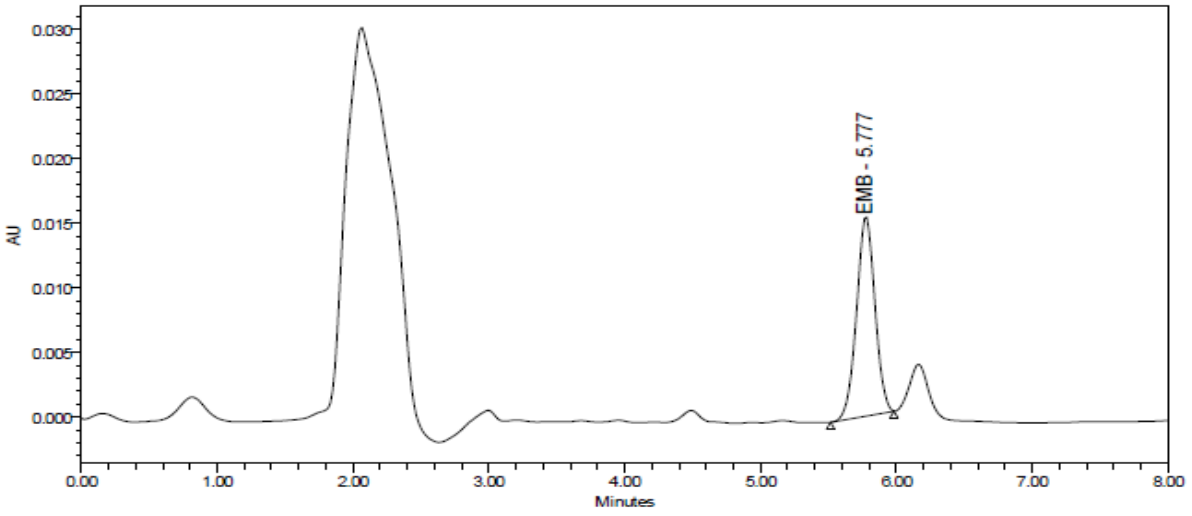
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.775	215095	100.00	22628	100.00	78.536

Fig. 19. Chromatogram of EMB in plasma at 1 h post dose of @ 50 mg kg^{-1} BW.



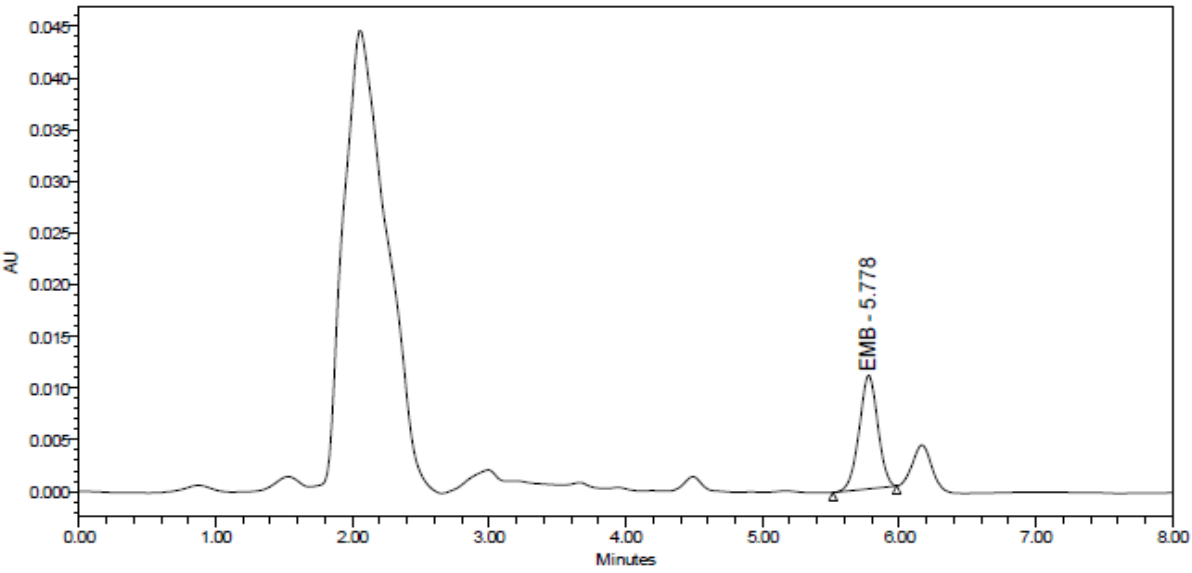
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.783	195330	100.00	20668	100.00	71.287

Fig. 20. Chromatogram of EMB in plasma at 2 h post dose of @ 50 mg kg^{-1} BW.



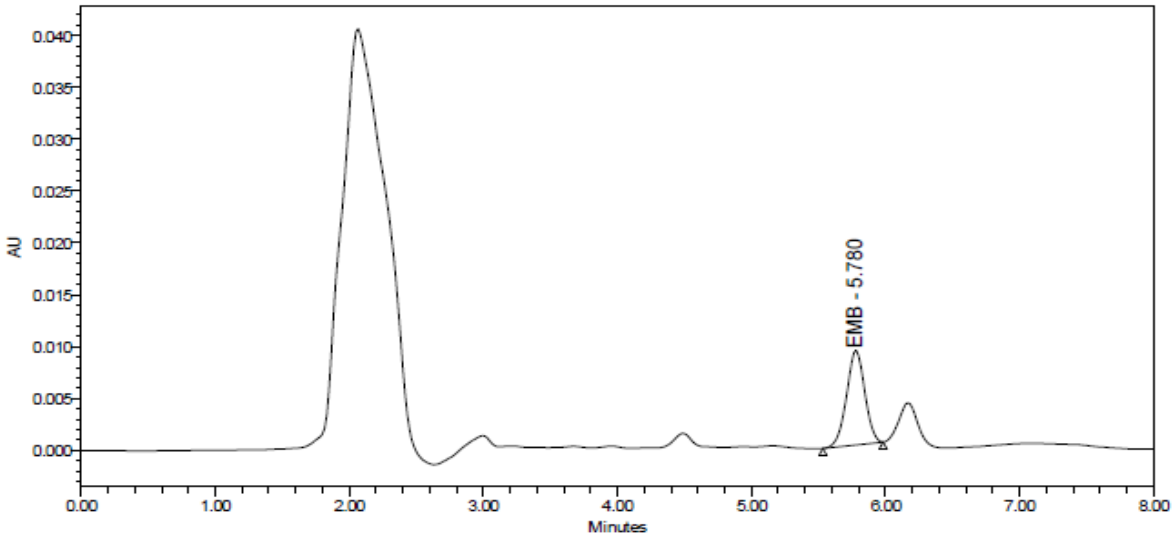
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.777	145441	100.00	15463	100.00	52.989

Fig. 21. Chromatogram of EMB in plasma at 4 h post dose of @ 50 mg kg^{-1} BW.



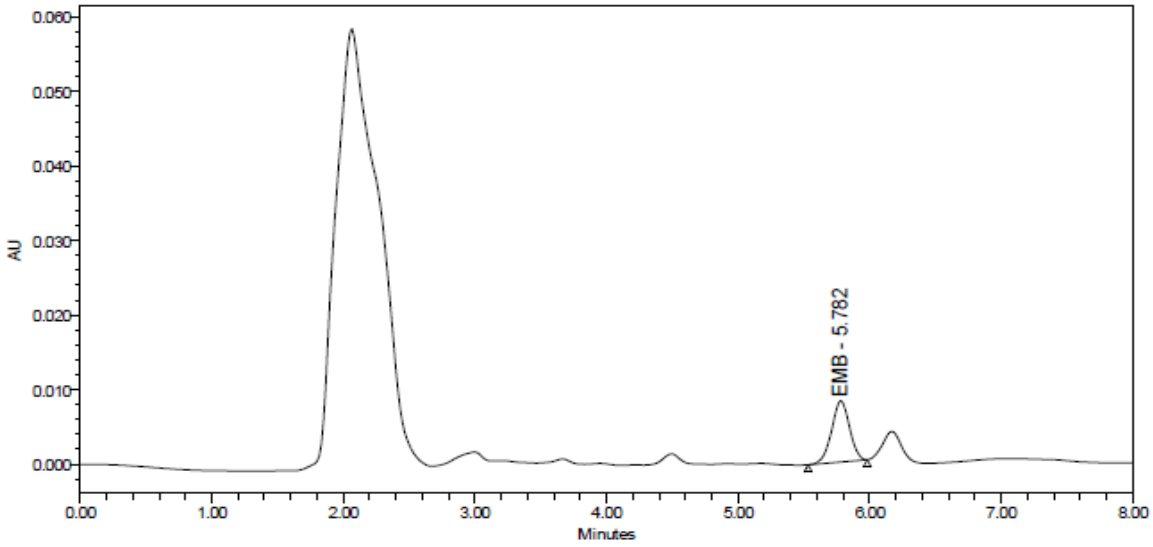
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.778	104277	100.00	11008	100.00	37.891

Fig. 22. Chromatogram of EMB in plasma at 8 h post dose of @ 50 mg kg^{-1} BW.



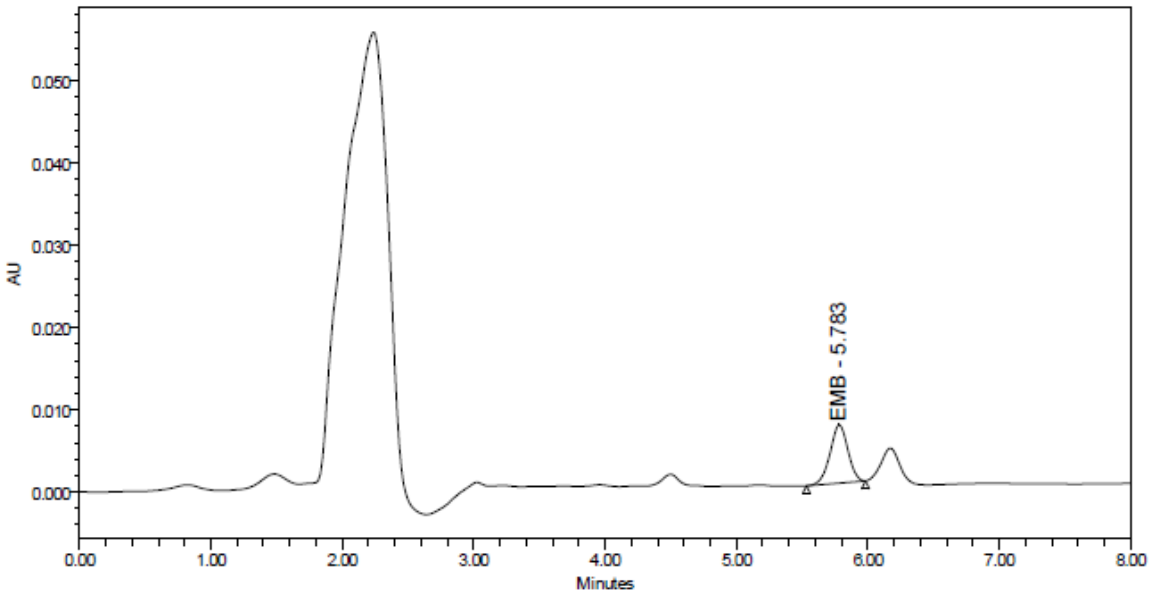
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.780	85887	100.00	9112	100.00	31.146

Fig. 23. Chromatogram of EMB in plasma at 12 h post dose of @ 50 mg kg^{-1} BW.



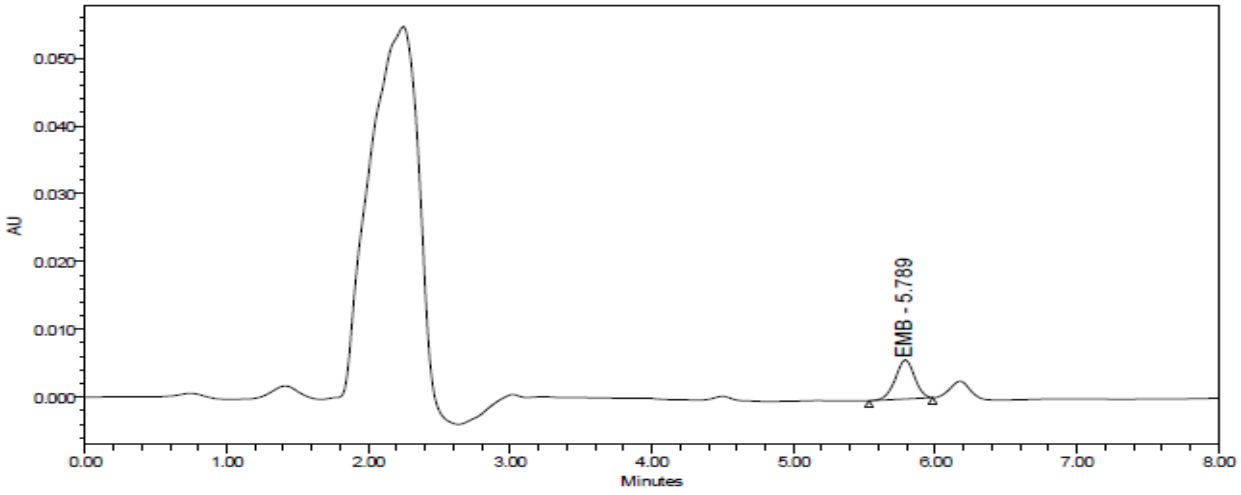
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.782	77516	100.00	8247	100.00	28.076

Fig. 24. Chromatogram of EMB in plasma at 24 h post dose of @ 50 mg kg^{-1} BW.



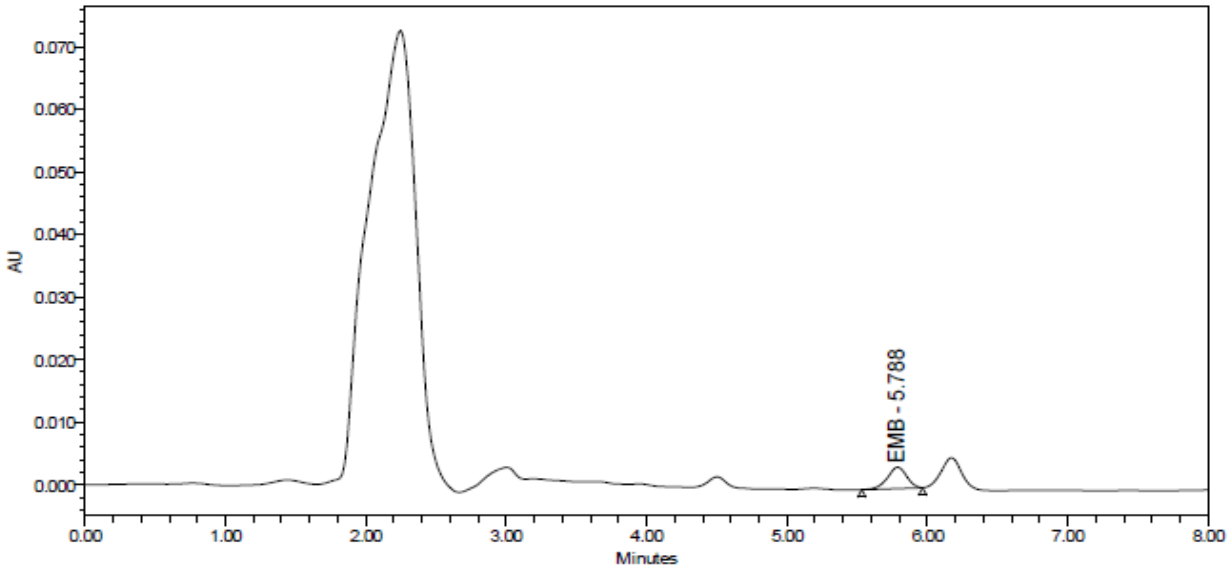
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.783	66545	100.00	7098	100.00	24.052

Fig. 25. Chromatogram of EMB in plasma at 36 h post dose of @ 50 mg kg^{-1} BW.



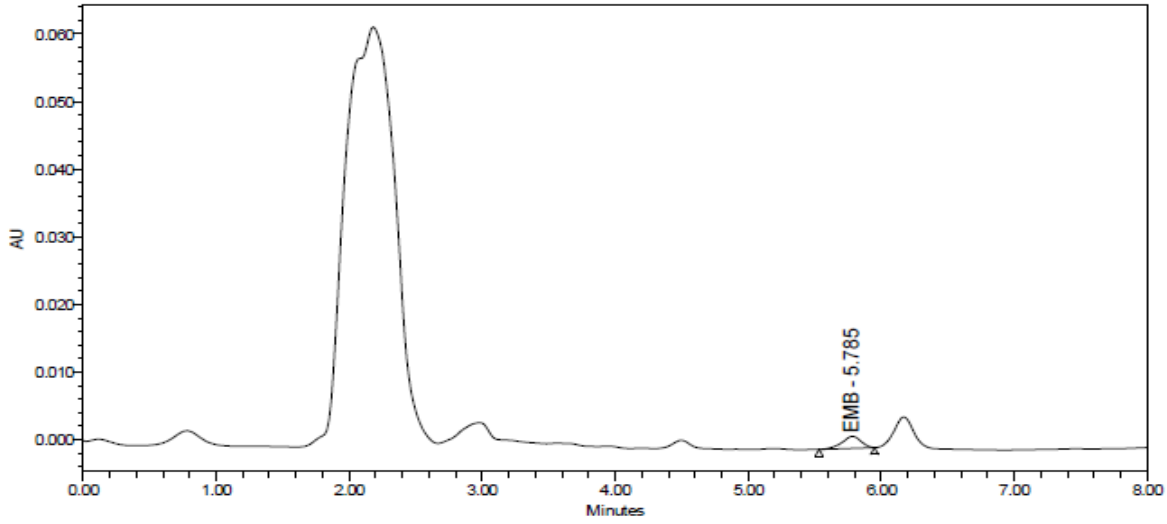
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.789	54332	100.00	5751	100.00	19.573

Fig. 26. Chromatogram of EMB in plasma at 48 h post dose of @ 50 mg kg^{-1} BW.



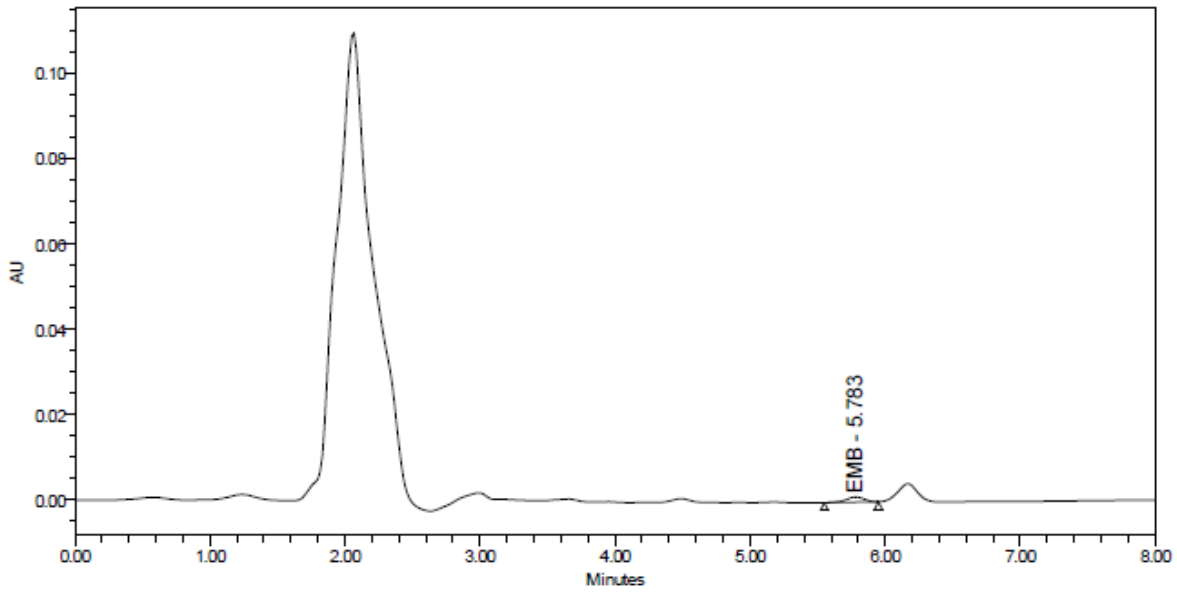
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.789	32137	100.00	3413	100.00	11.432

Fig. 27. Chromatogram of EMB in plasma at 72 h post dose of @ 50 mg kg^{-1} BW.



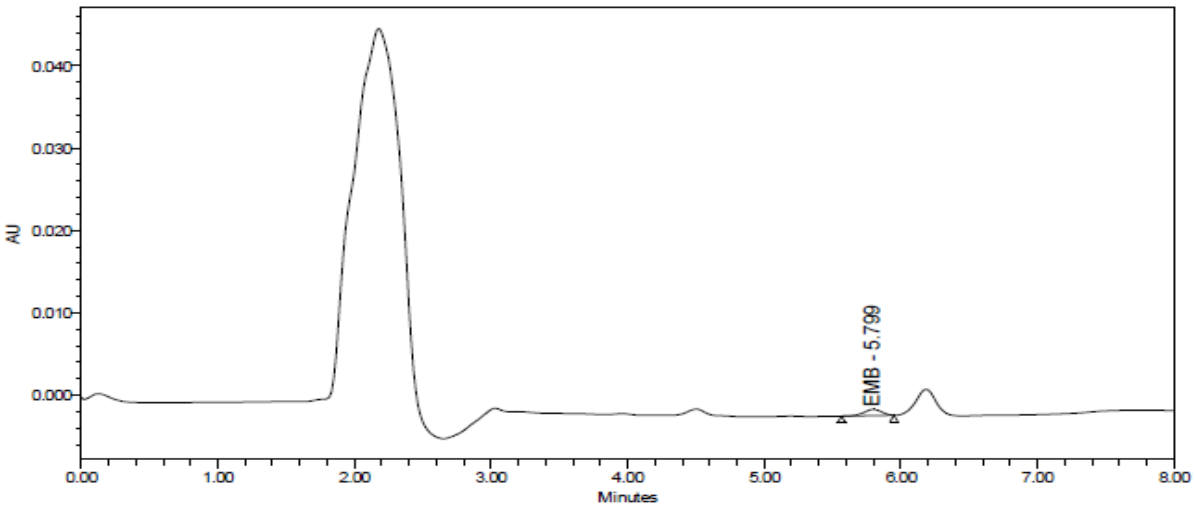
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.785	17524	100.00	1778	100.00	6.073

Fig. 28. Chromatogram of EMB in plasma at 96 h post dose of @ 50 mg kg^{-1} BW.



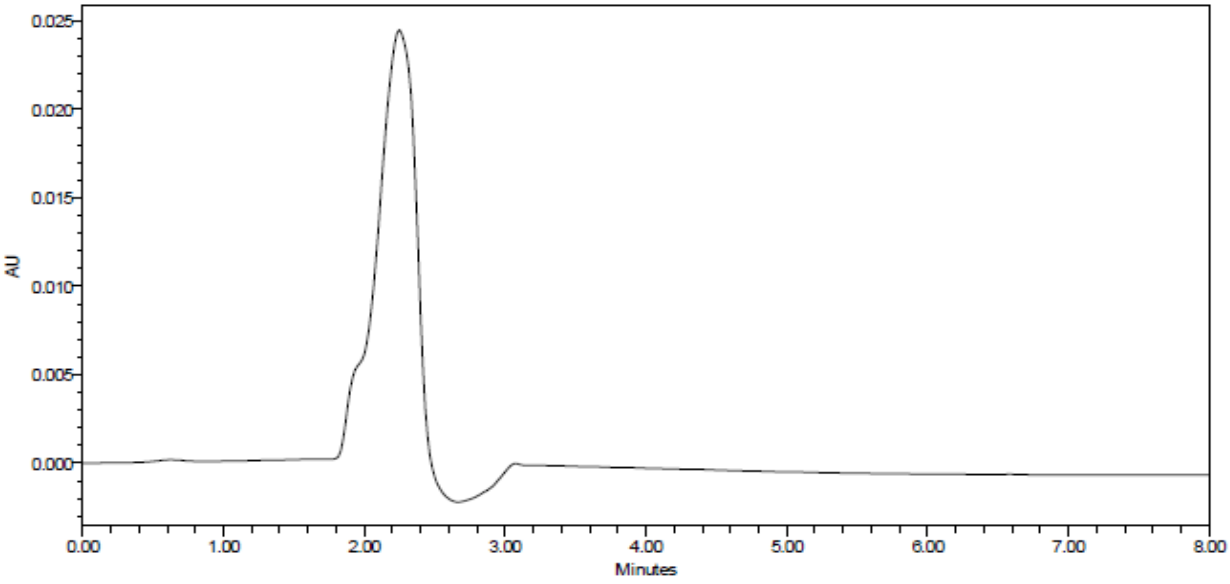
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.783	11048	100.00	1214	100.00	3.698

Fig. 29. Chromatogram of EMB in plasma at 120 h post dose of @ 50 mg kg^{-1} BW.



Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.799	6687	100.00	752	100.00	2.098

Fig. 30. Chromatogram of EMB in plasma at 144 h post dose of @ 50 mg kg^{-1} BW.



Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.730	0	0	0	0	0

Fig. 31. Chromatogram of EMB in plasma at 168 h post dose of @ 50 mg kg^{-1} BW.

Table. 7. Plasma concentration of EMB after single dose @ 20 mg kg^{-1} body weight through the feed.

Time after drug administration (h)	The concentration of EMB (µg ml^{-1})
0.5	$10.32^{d} \pm 0.01$
1	$21.82^{a} \pm 0.01$
2	$17.09^{b} \pm 0.01$
4	$11.65^{c} \pm 0.00$
8	$5.90^{e} \pm 0.00$

12	$2.25^{f} \pm 0.00$
24	$1.28^{g} \pm 0.00$
36	$0.8^{h} \pm 0.00$
48	$0.67^{i} \pm 0.00$
72	$0.62^{j} \pm 0.00$
96	$0.41^{k} \pm 0.00$
120	ND

Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

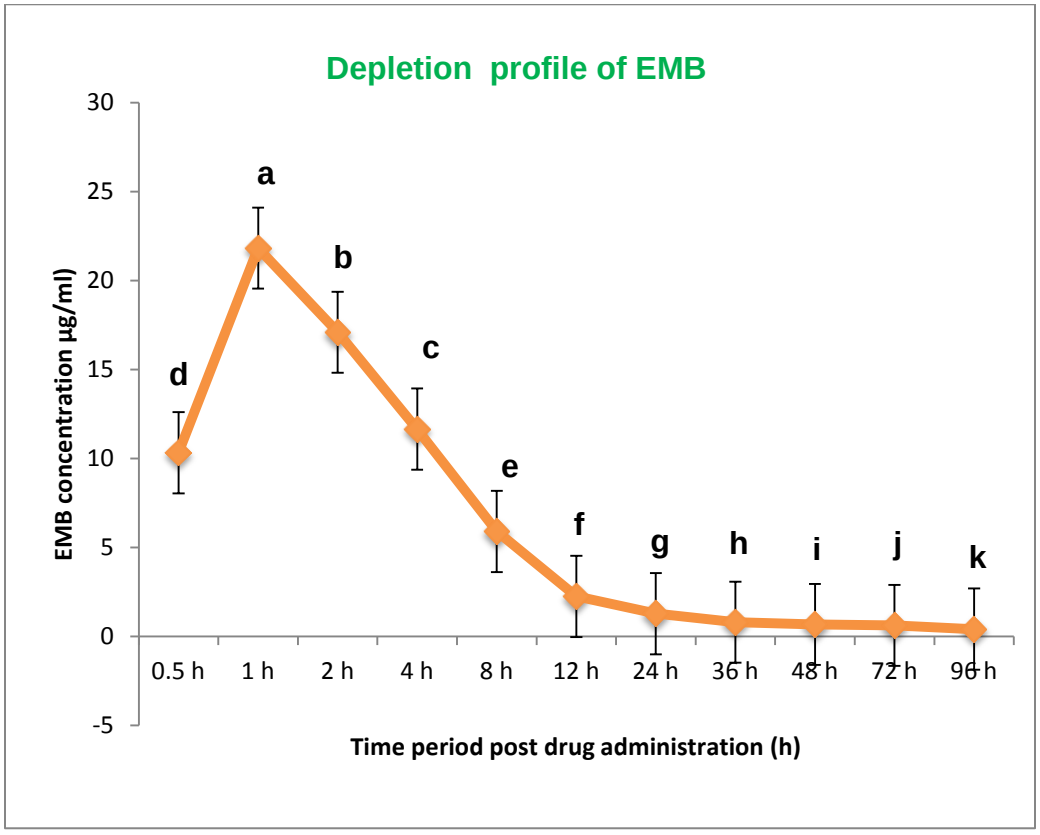
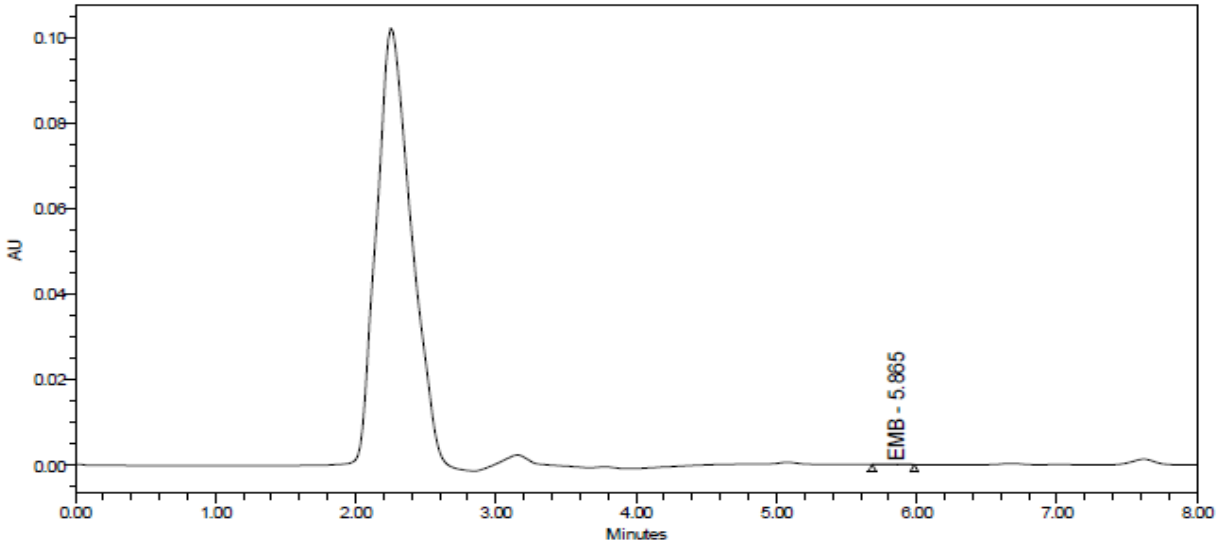
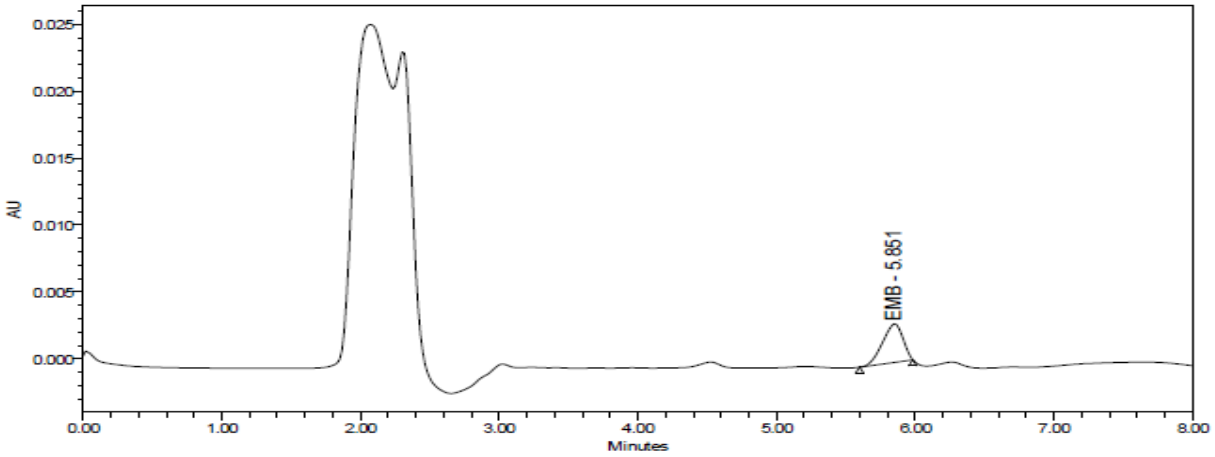


Fig. 32. Depletion profile of plasma EMB concentration after oral administration of @ 20 mg kg^{-1} BW through the feed.



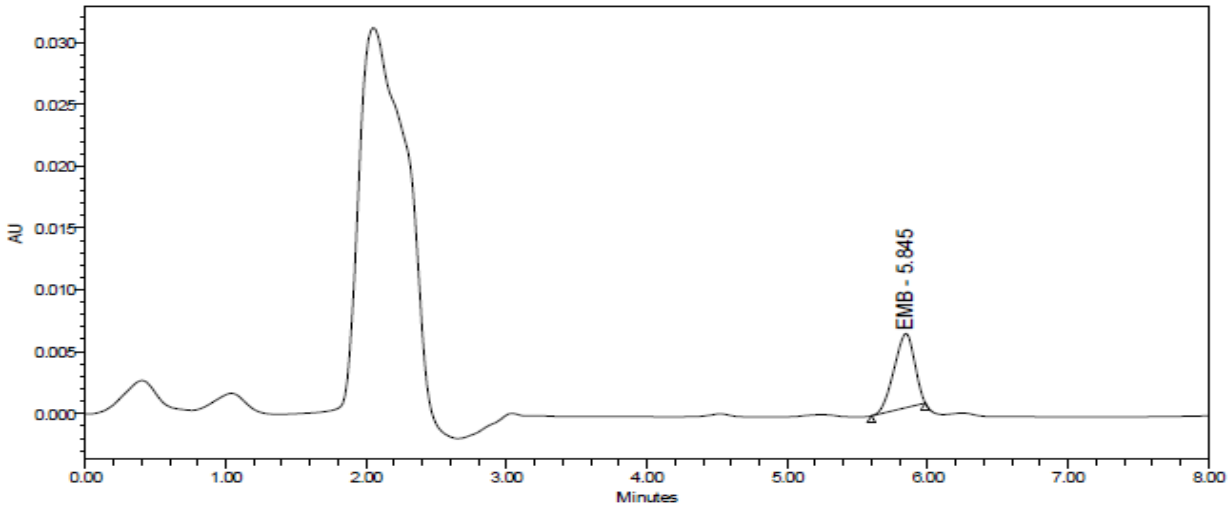
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.865	414	100.00	50	100.00	0

Fig. 33. Chromatogram of EMB in plasma at 0 h post dose of @ 20 mg kg^{-1} BW through the feed.



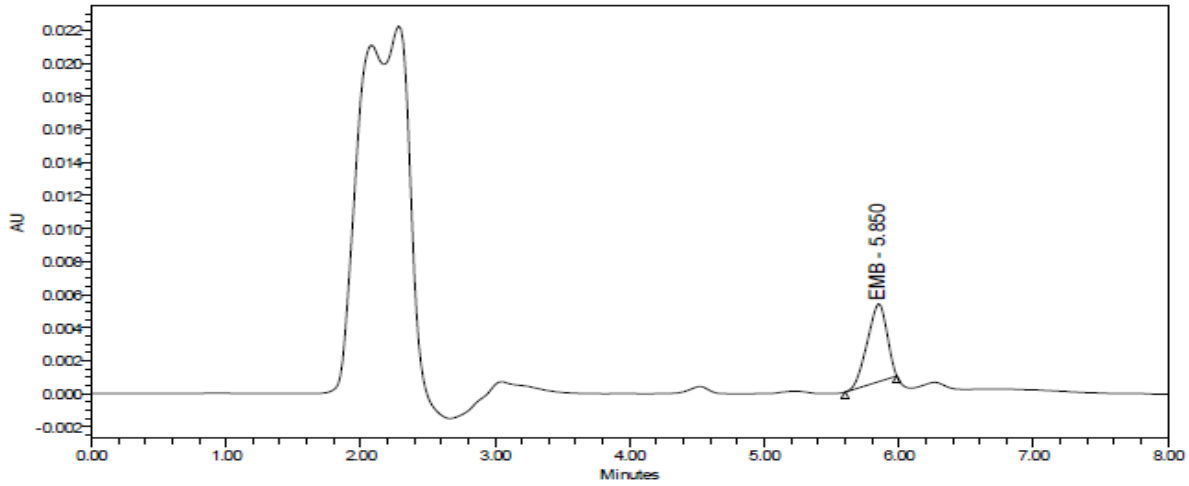
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.851	29202	100.00	2886	100.00	10.356

Fig. 34. Chromatogram of EMB in plasma at 0.5 h post dose of @ 20 mg kg^{-1} BW through the feed.



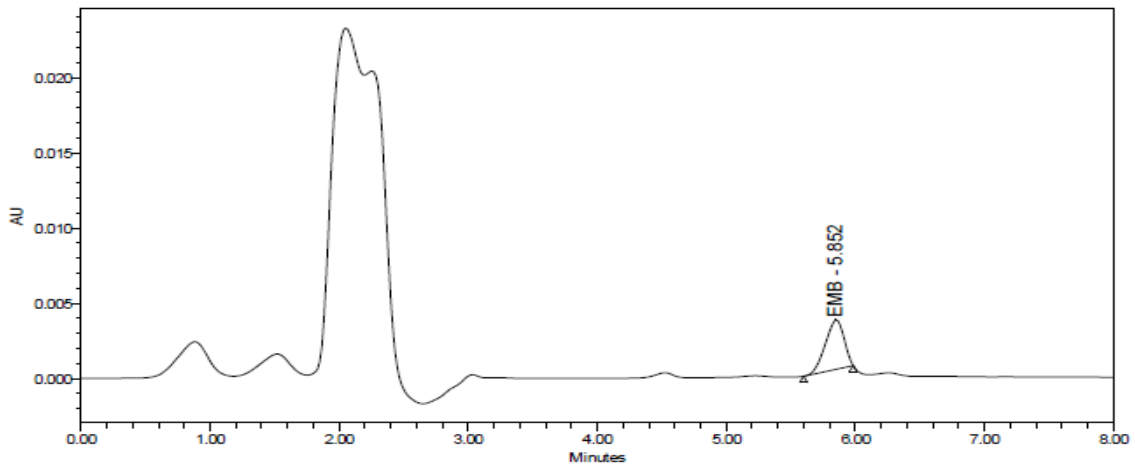
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.845	60512	100.00	6020	100.00	21.839

Fig. 35. Chromatogram of EMB in plasma at 1 h post dose of @ 20 mg kg^{-1} BW through the feed.



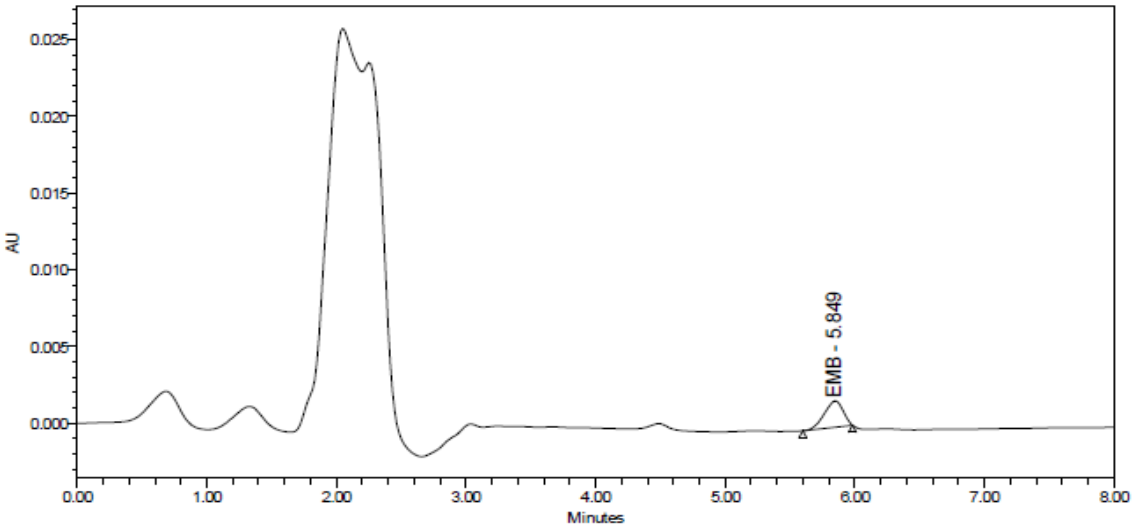
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.850	47492	100.00	4716	100.00	17.064

Fig. 36. Chromatogram of EMB in plasma at 2 h post dose of @ 20 mg kg^{-1} BW through the feed.



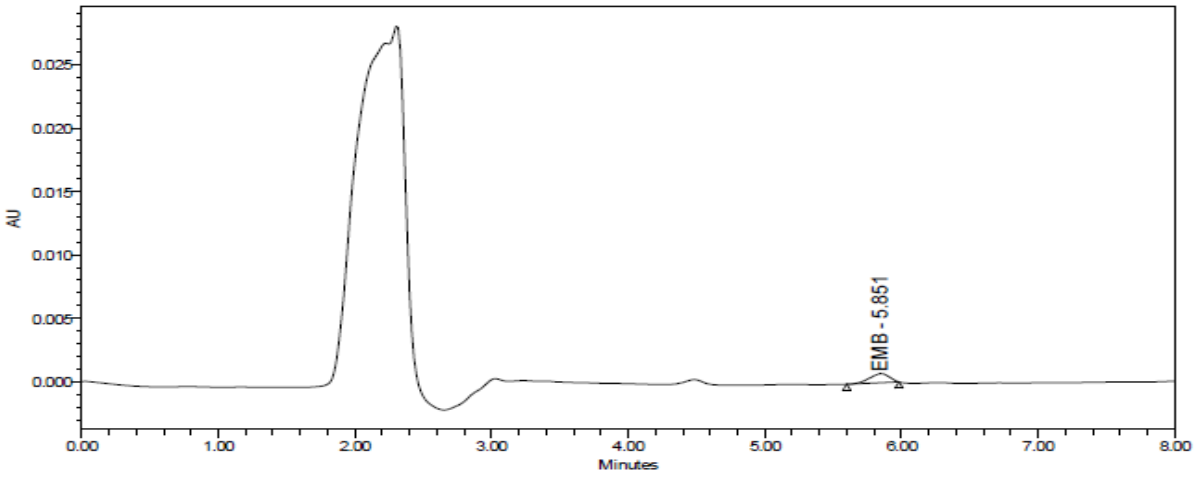
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.852	32737	100.00	3293	100.00	11.652

Fig. 37. Chromatogram of EMB in plasma at 4 h post dose of @ 20 mg kg^{-1} BW through the feed.



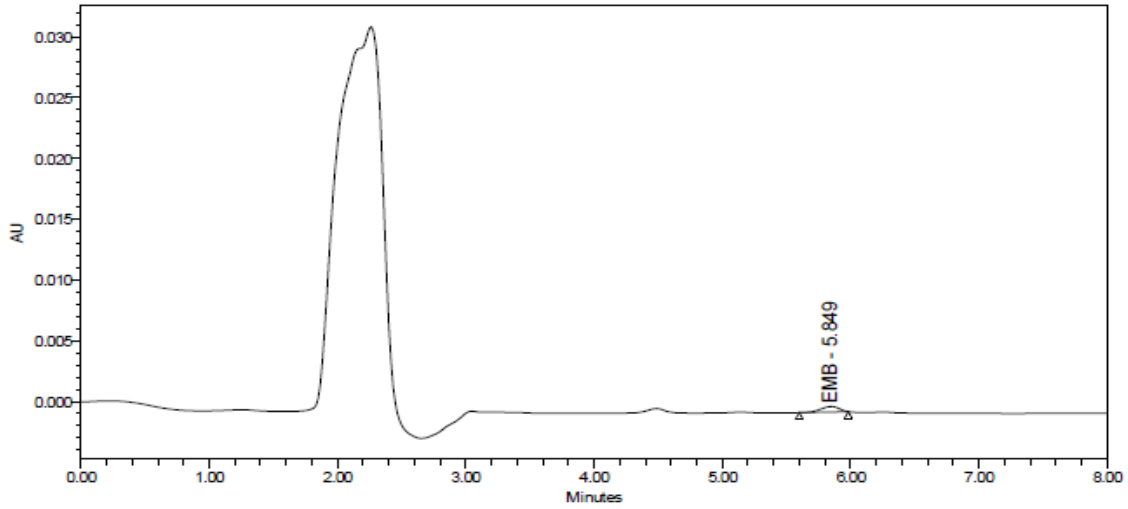
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.849	17086	100.00	1722	100.00	5.912

Fig. 38. Chromatogram of EMB in plasma at 8 h post dose of @ 20 mg kg^{-1} BW through the feed.



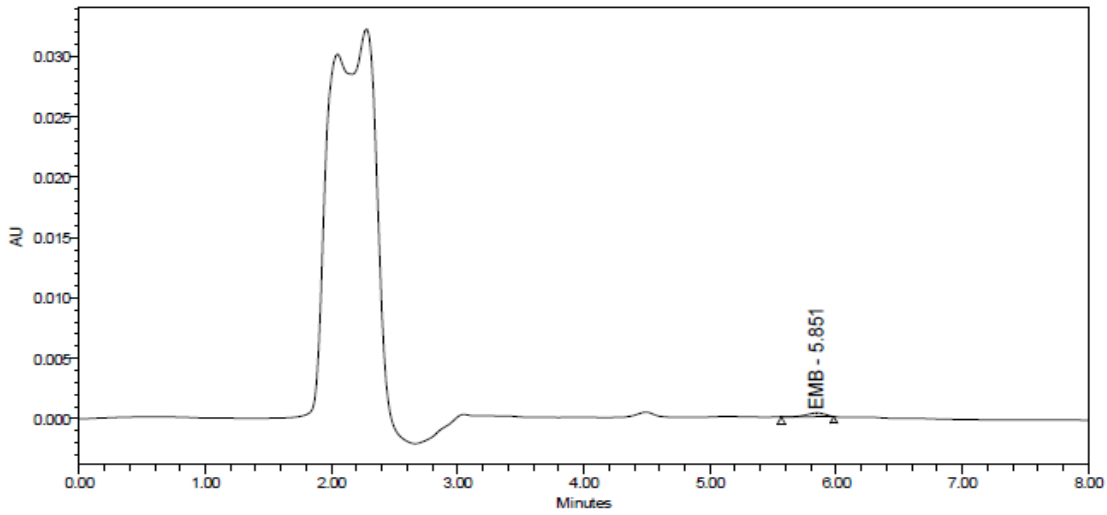
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.851	7118	100.00	715	100.00	2.256

Fig. 39. Chromatogram of EMB in plasma at 12 h post dose of @ 20 mg kg^{-1} BW through the feed.



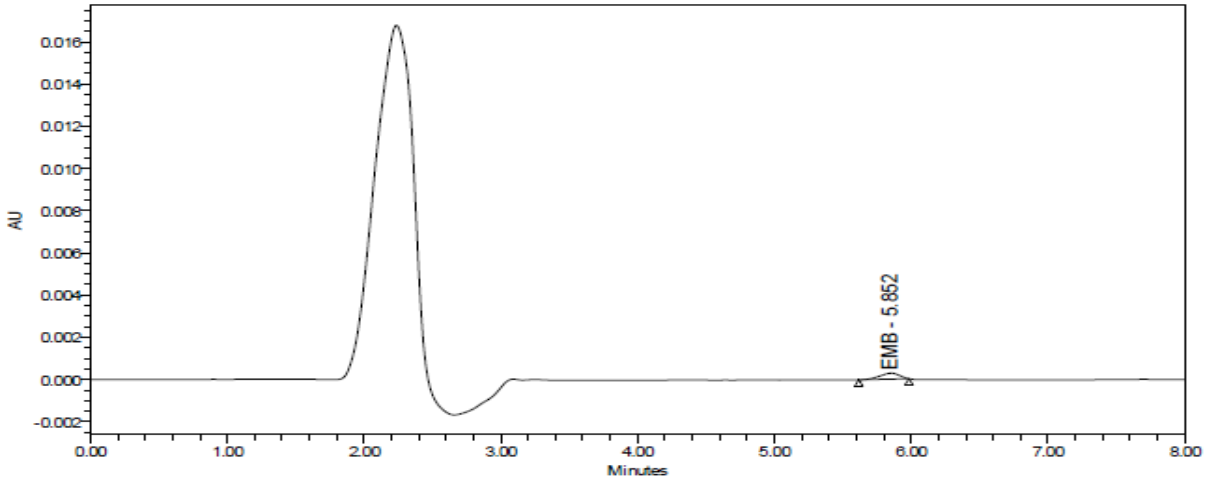
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.849	4477	100.00	457	100.00	1.287

Fig. 40. Chromatogram of EMB in plasma at 24 h post dose of @ 20 mg kg^{-1} BW through the feed.



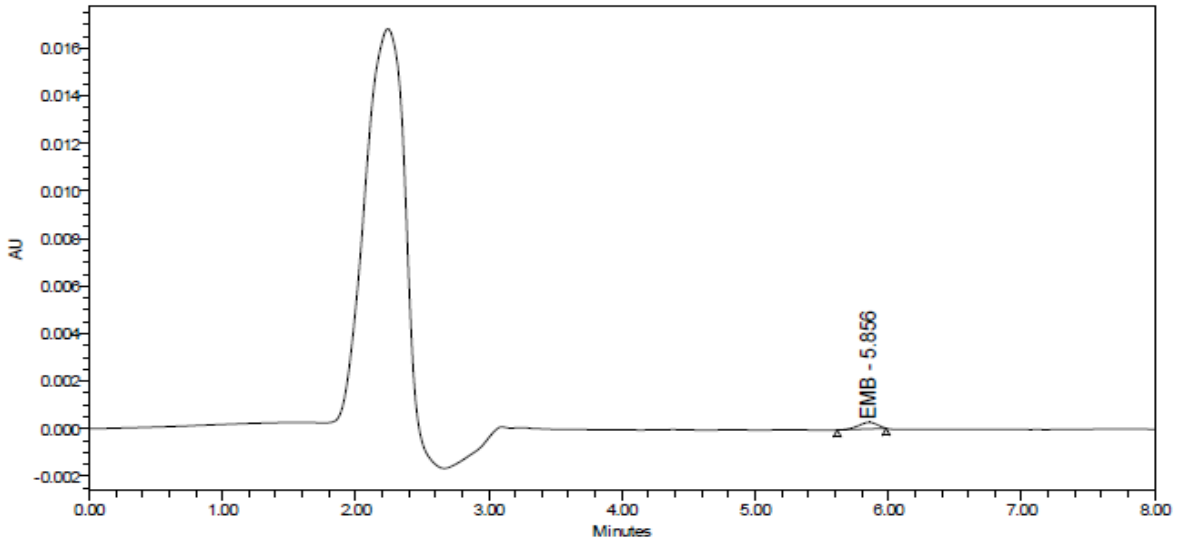
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.851	3148	100.00	320	100.00	0.800

Fig. 41. Chromatogram of EMB in plasma at 36 h post dose of @ 20 mg kg^{-1} BW through the feed.



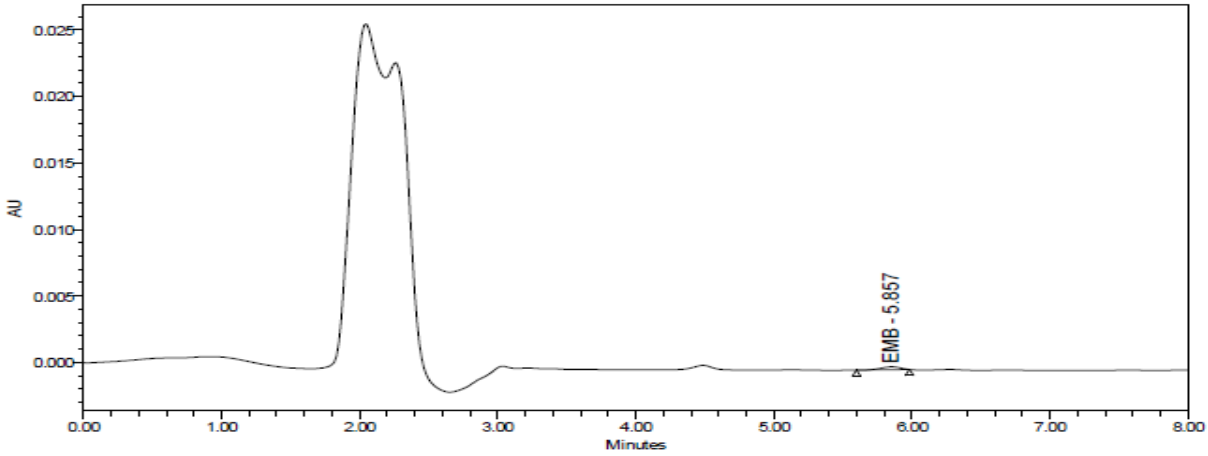
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.852	2818	100.00	287	100.00	0.679

Fig. 42. Chromatogram of EMB in plasma at 48 h post dose of @ 20 mg kg^{-1} BW through the feed.



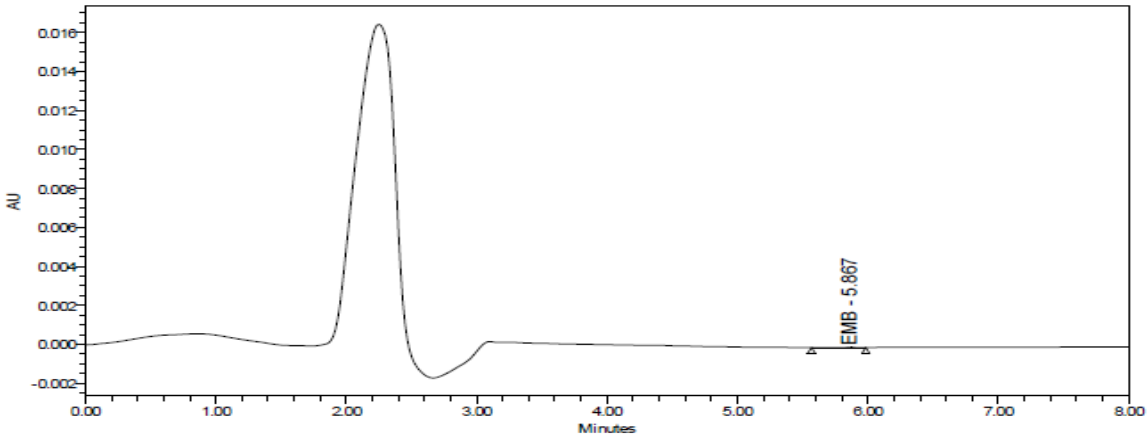
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.856	3148	100.00	279	100.00	0.626

Fig. 43. Chromatogram of EMB in plasma at 72 h post dose of @ 20 mg kg^{-1} BW through the feed.



Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.856	2107	100.00	221	100.00	0.418

Fig. 44. Chromatogram of EMB in plasma at 96 h post dose of @ 20 mg kg^{-1} BW through the feed.



Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.856	55	100.00	5	100.00	0

Fig. 45. Chromatogram of EMB in plasma at 120 h post dose of @ 20 mg kg^{-1} BW through the feed.

4.3. Bioaccumulation of EMB residue in muscle tissue.

The bioaccumulation of drug residues in muscle tissue of *Labeo rohita* was analyzed at different days. The drug residue was detected on the 3, 6 and 9 day. It ensures that there was completely eliminated the drug residues from *Labeo rohita* body by the 12 days. The concentration of EMB in muscles presented in table 8 and Figure 46 to 51.

Table.8. Bioaccumulation of EMB in muscle tissues of *L. rohita* after a single dose of @ 20 mg kg^{-1} body weight through the feed.

Treatment (days)	EMB (µg ml^{-1})
0	ND
3	0.38^{b}±0.00
6	0.83^{a}±0.00
9	0.23^{c}±0.03

12	ND
15	ND

Each time point (n=3) represent the mean ± SE; Bars with different letters represent significant differences ($p<0.05$).

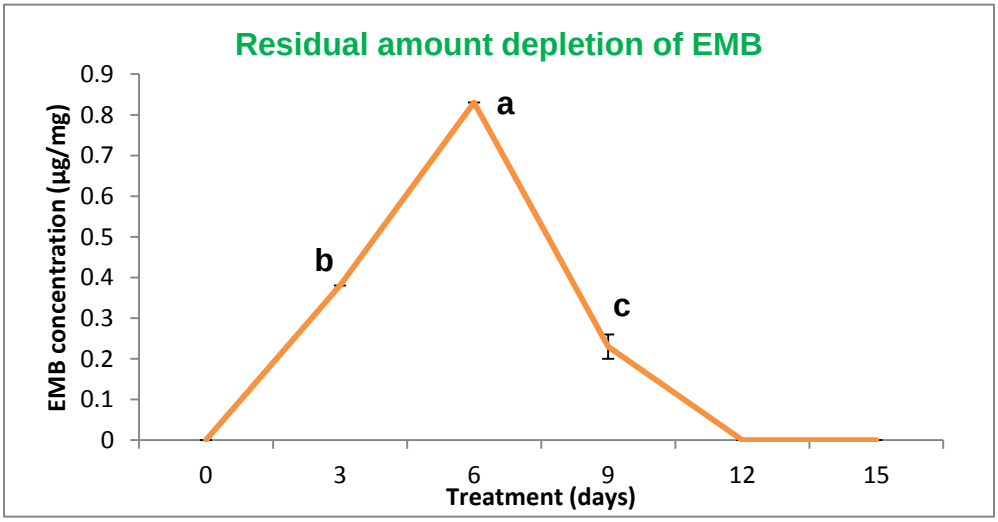
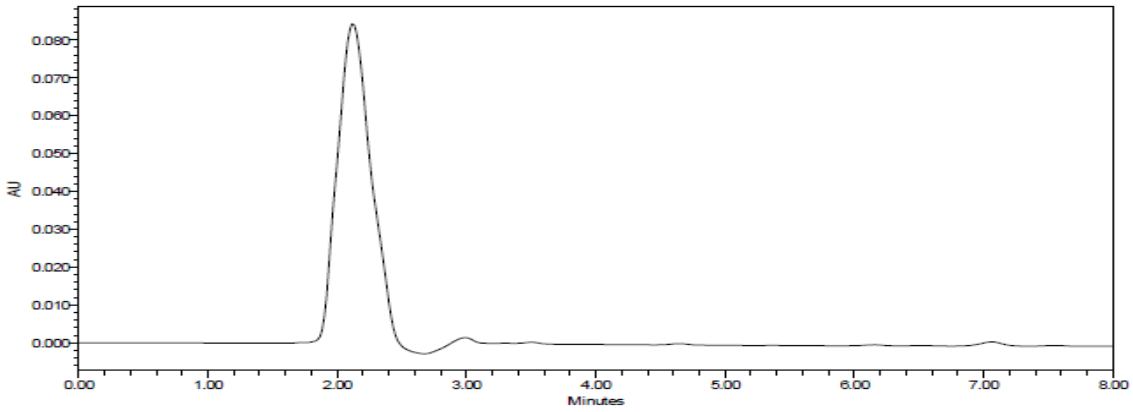
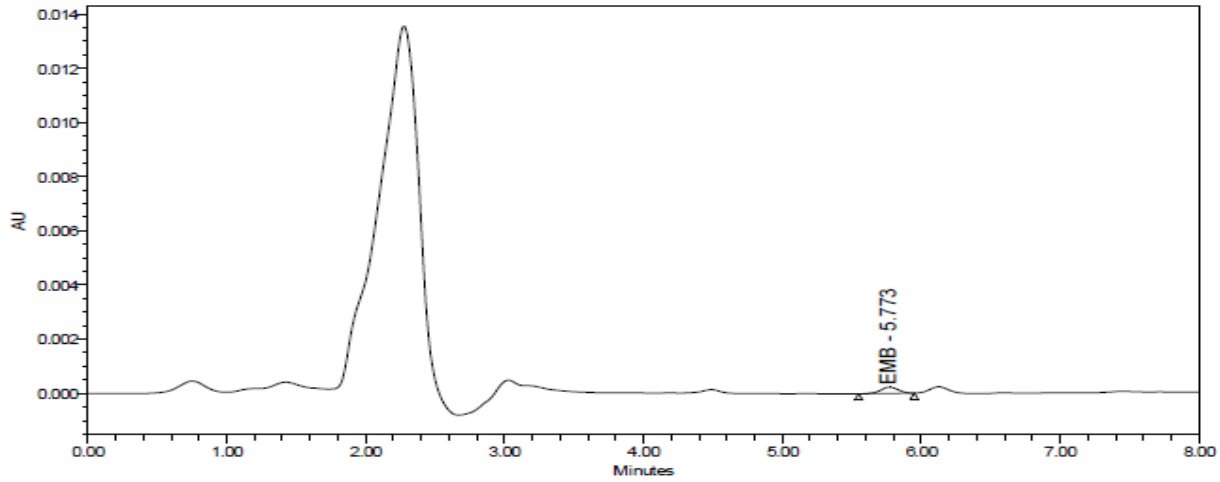


Fig. 46. Residual amount depletion of EMB in the muscle



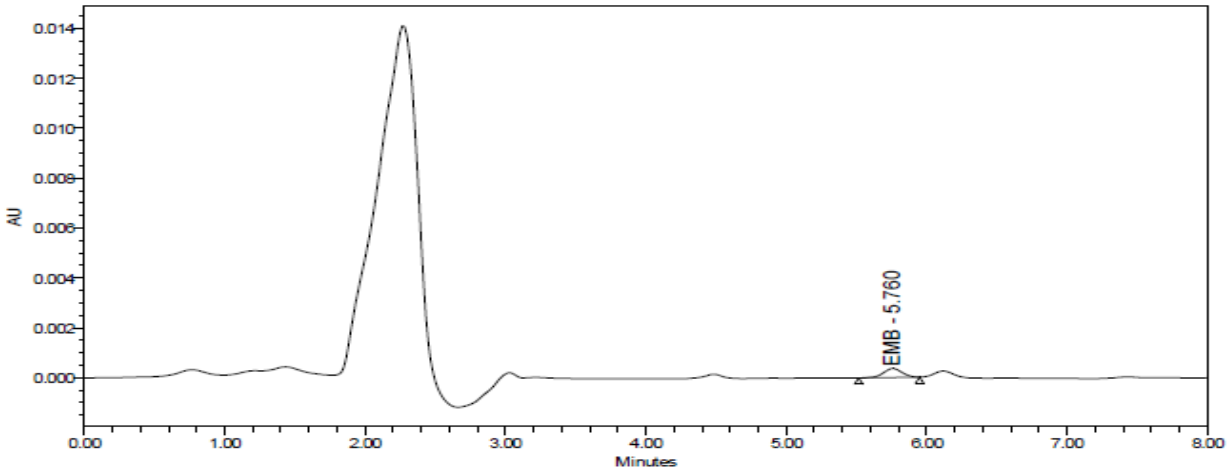
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.730	0	0	0	0	0

Fig. 47. Chromatogram of EMB in muscle tissue at 0 day post dose of @ 20 mg kg^{-1} BW through the feed



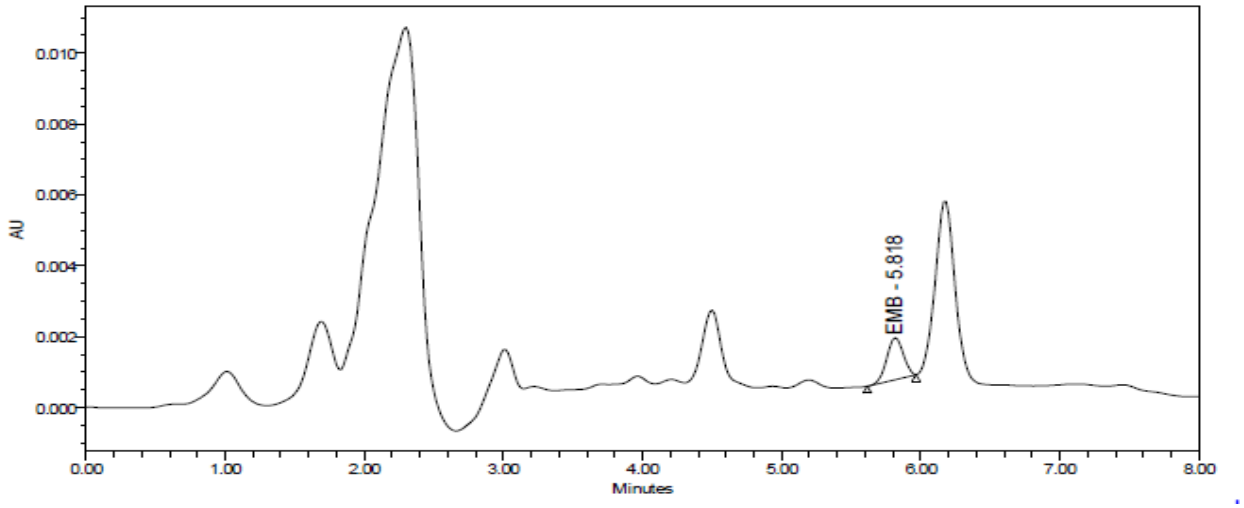
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.773	2056	100.00	232	100.00	0.399

Fig. 48. Chromatogram of EMB in muscle tissue at 3 day post dose of @ 20 mg kg^{-1} BW through the feed



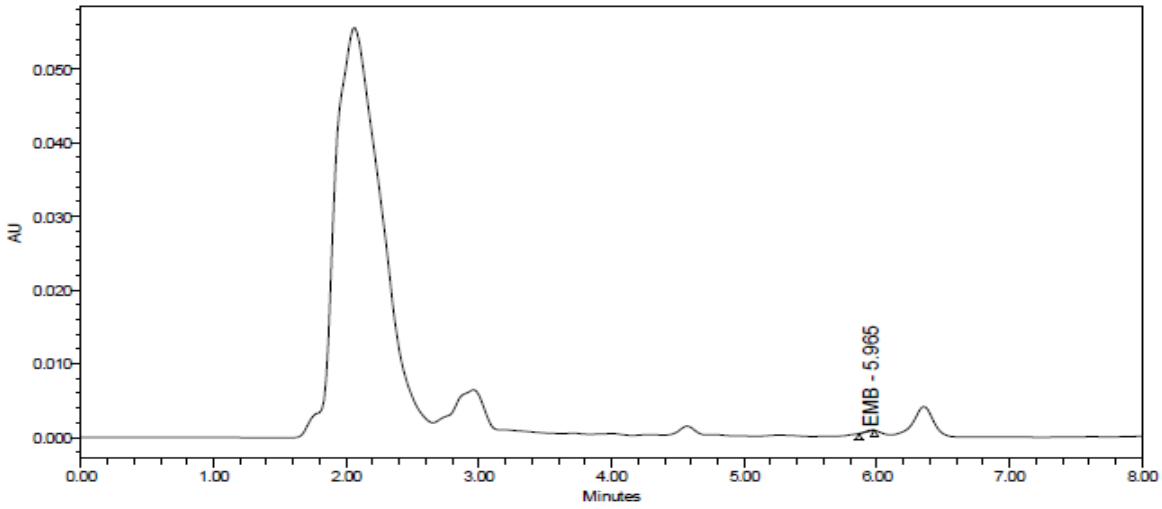
Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.760	3251	100.00	360	100.00	0.838

Fig. 49. Chromatogram of EMB in muscle tissue at 6 day post dose of @ 20 mg kg^{-1} BW through the feed



Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.760	9759	100.00	1184	100.00	0.200

Fig. 50. Chromatogram of EMB in muscle tissue at 9 day post dose of @ 20 mg kg^{-1} BW through the feed



Peak Name	RT(Min)	Area(µV*Sec)	% Area	Height(µV)	% Height	Amount
EMB	5.965	503	100.00	104	100.00	0

Fig. 51. Chromatogram of EMB in muscle tissue at 12 day post dose of @ 20 mg kg^{-1} BW through the feed

4.4. Proximate composition of diets

The experimental diets were prepared to contain 32.09% crude protein and 6% lipid. After analysis, it was found that the crude protein of the diets varied from 32.7± 0.12% to 33.1± 0.12% whereas the ether extract level varied from 5.6 to 5.9%. The ash content of the diets varied from 9 to 9.2%. The crude fiber of the diets was estimated in the range of 0.8 to 4.7%. The calculated nitrogen-free extract was ranged from 44 to 46%. The calculated gross energy (GE) varied from 396.47 to 398.2 Kcal/100g feed. The proximate composition of the diets is shown in table 9.

Table. 9. Proximate composition of the control and medicated feed.

Treatment	Moisture	Crude protein	Ether extract	Crude fibre	NFE	Gross energy (kcal/100g)	Ash
Control feed	7.6±0.30	32.03±0.12	5.8±0.03	0.8±0.04	44.6±2.3	396.7±5.0	9.1±0.12
Medicated feed	7.2±0.09	33.1± 0.12	5.7±0.12	0.6±0.02	45.3±3.5	398.2±8.7	9.2±0.4

Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$)

Calculated digestible energy (Kcal/100g) = (%CP×4) + (%EE×9) + (%NFE×4) Halver, 1976.

4.5. Enzyme assay

4.5.1. Aspartate aminotransaminase (AST)

AST activity of *Labeo rohita* juveniles was estimated at 0,3, 6, 9, 12 and 15 days interval in serum after post-feeding of EMB @ 20 mg kg^{-1} body weight and the results are shown in table 10 and figure 52, respectively. The activity of the AST in

serum in different days differ significantly ($p< 0.05$). The AST activity was the highest in serum on the 6 days (22.13 ± 0.37).

Table. 10. Day wise change in AST activity in serum of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

Treatment (days)	IU L^{-1}
0	10.12^{d} ± 0.64
3	15.12^{b} ± 0.37
6	22.13^{a} ± 0.37
9	16.30^{b} ± 0.99
12	12.00^{c} ± 0.37
15	10.50^{cd} ± 0.37

Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

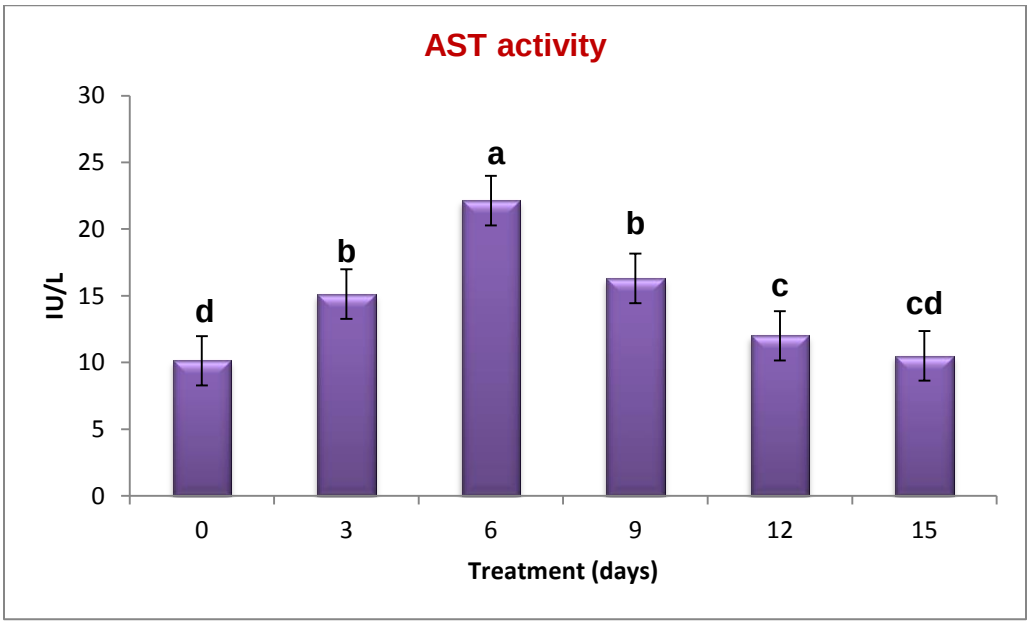


Fig. 52. Day wise change in AST activity in serum of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

4.5.2. Alanine aminotransaminase (ALT)

ALT activity of *Labeo rohita* juveniles was estimated at 0, 3, 6, 9, 12 and 15 days interval in serum after post feeding with EMB @ 20 mg kg^{-1} body weight and the results are shown in table 11 and figure 53, respectively. The activity of the ALT in serum in different days differ significantly ($p< 0.05$). The ALT activity in serum varies from 12.00 ± 0.75 on the 0 days to highest on 6 days 27.00 ± 0.00 and reached up to 10.87 ± 1.63 (normal level) on 15 days.

Table. 11. Day wise change in ALT activity in serum of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

Treatment (days)	IU L^{-1}
0	12.00^{d} ± 0.75
3	23.25^{b} ± 0.75
6	27.00^{a} ± 0.00
9	21.38^{bc} ± 0.64
12	19.13^{c} ± 0.64
15	10.87^{d} ± 1.63

Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

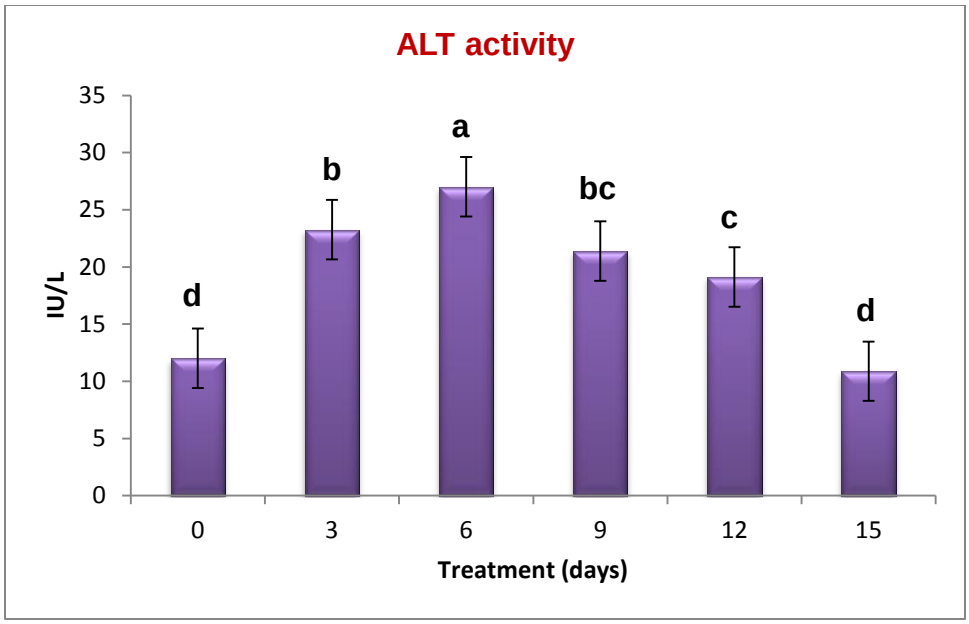


Fig. 53. Day wise change in ALT activity in serum of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

4.5.3. SOD (Superoxide dismutase)

SOD activity of *Labeo rohita* juveniles was estimated at 0, 3, 6, 9, 12, and 15 days interval in liver and gill after post feeding with EMB @ 20 mg kg^{-1} body weight to *Labeo rohita* and results shown in table 12 and figure 54 & 55, respectively. The activity of the SOD enzyme was found significantly different ($p<0.05$) in the different experimental days. The SOD activity was highest at 6 days in both liver (29.21 ± 0.71) and gill (24.39 ± 0.15).

Table. 12. SOD activity in liver and gill of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

Treatment (days)	Liver	Gill
0	$11.28^{e} \pm 0.32$	$9.47^{e} \pm 1.24$
3	$23.04^{b} \pm 0.45$	$15.27^{d} \pm 0.21$
6	$29.21^{a} \pm 0.71$	$24.39^{a} \pm 0.15$

9	$20.42^{c} \pm 0.79$	$20.34^{b} \pm 0.22$
12	$15.12^{d} \pm 0.14$	$17.28^{c} \pm 0.33$
15	$11.82^{e} \pm 0.63$	$9.35^{e} \pm 0.60$

SOD activity is expressed as units- one unit is 50% inhibition of epinephrine auto-oxidation min^{-1}mg $protein^{-1}$. Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

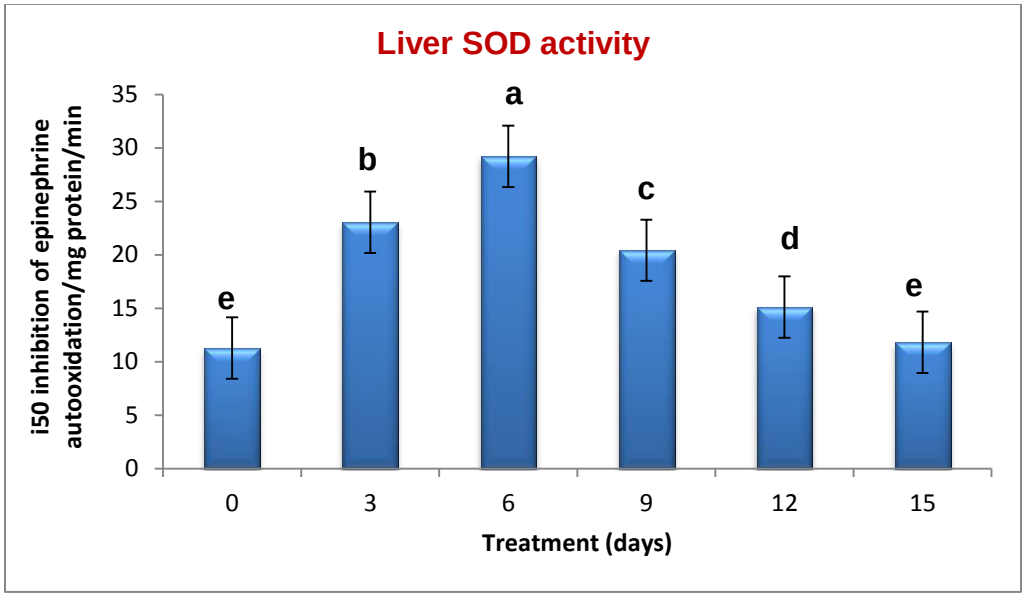


Fig. 54. Day wise change in SOD activity in liver of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

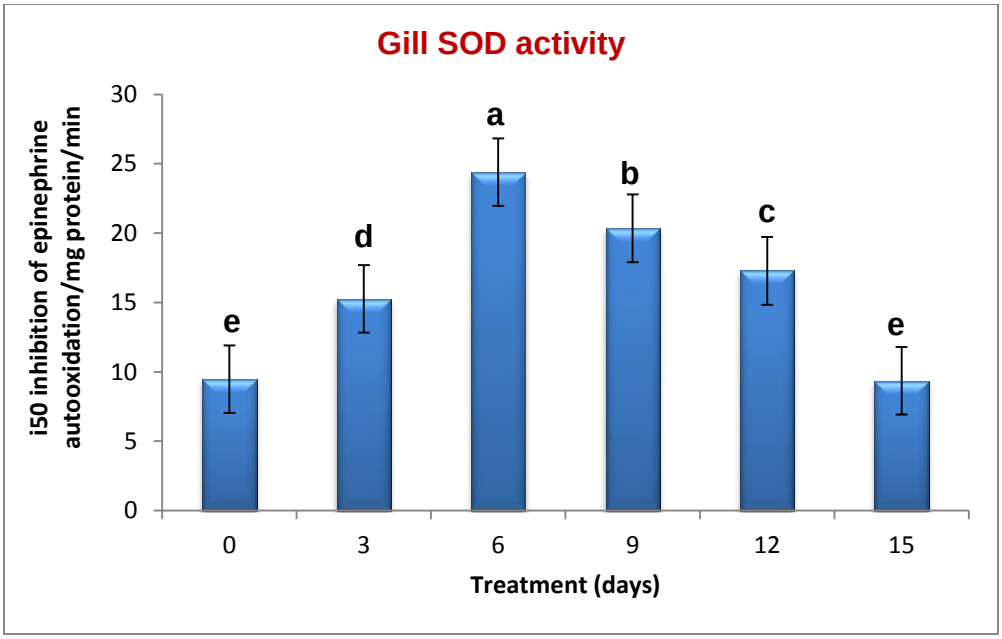


Fig. 55. Day wise change in SOD activity in gill of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

4.5.4. Catalase (CAT)

Catalase activity of *Labeo rohita* juveniles was estimated at 0, 3, 6, 9, 12, and 15 days interval in liver and gill after post feeding with EMB @ 20 mg kg^{-1} body weight to *Labeo rohita* and results shown in table 13 and figure 56 & 57 respectively. The activity of the catalase enzyme found significantly different ($p<0.05$) in different experimental days. The catalase activity was highest at 6 days in both liver (23.51 ± 1.71) and gill (20.99 ± 0.32).

Table. 13. Day wise change in CAT activity in liver and gill of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

Treatment (days)	Liver	Gill
0	10.19^{d} ± 0.50	11.70^{d} ± 0.23
3	12.61^{d} ± 0.23	16.53^{bc} ± 1.28
6	23.51^{a} ± 1.71	20.99^{a} ± 0.32

9	$18.84^{b} \pm 0.68$	$18.34^{b} \pm 0.46$
12	$15.82^{c} \pm 1.31$	$15.06^{c} \pm 0.27$
15	$10.27^{d} \pm 0.49$	$11.41^{d} \pm 0.09$

CAT activity expressed as unit nanomoles H_2O_2 decomposed min^{-1}mg $protein^{-1}$. Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

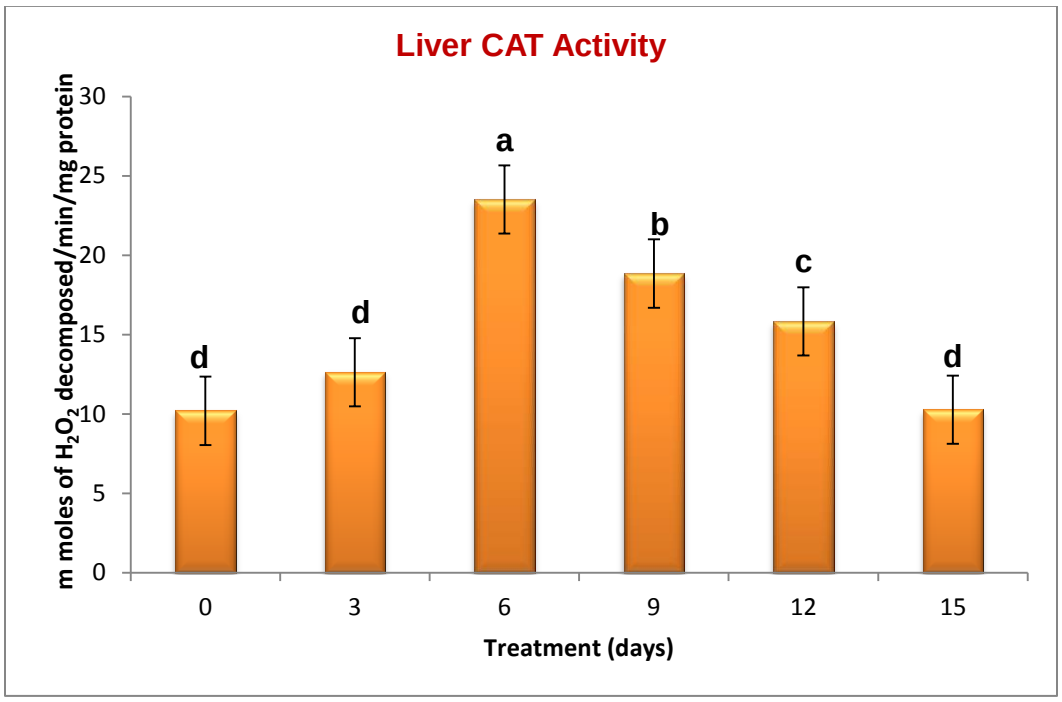


Fig. 56. CAT activity in liver of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

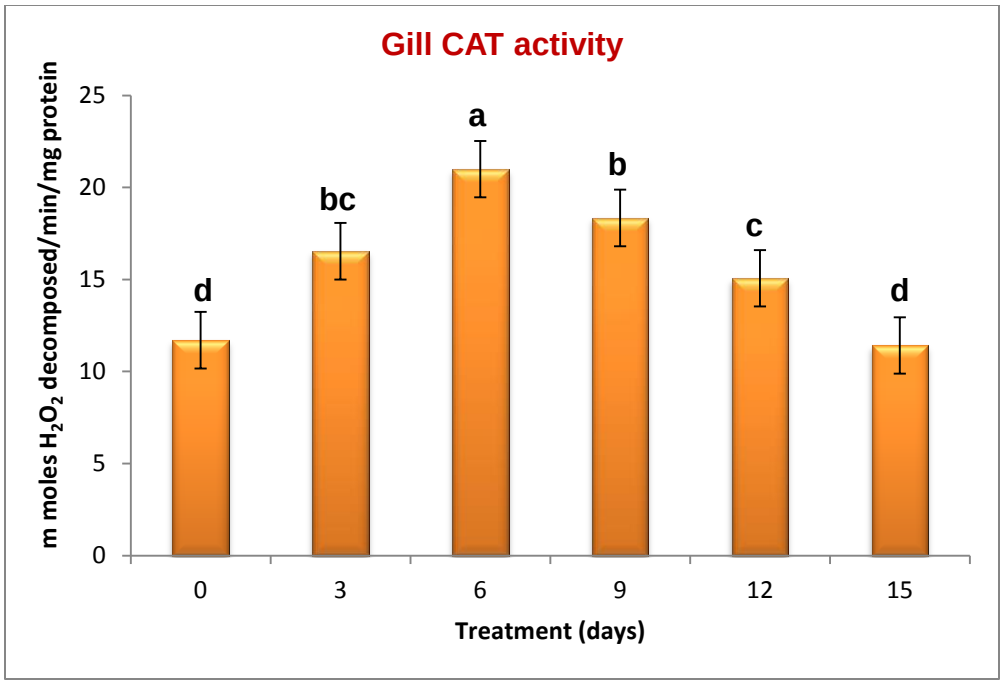


Fig. 57. CAT activity in gill of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

4.5.5. Glutathione S-transferase (GST) activity

The Glutathione S-transferases (GST) activity of *Labeo rohita* juveniles was estimated at 0, 3, 6, 9, 12 and 15 days interval in the liver after post-feeding of EMB @ 20 mg kg^{-1} body *Labeo rohita* and results shown in table 14 and figure 58. The activity of the GST varied significantly ($p<0.05$) among different days. The GST activity of liver was highest at 6 days (21.86 ± 0.29) post administered.

Table. 14. GST activity in the liver of *L. rohita*, fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

Treatment (days)	Liver
0	10.97^{d} ± 0.32
3	16.01^{c} ± 0.19
6	21.86^{a} ± 0.29
9	19.63^{b} ± 0.10

12	$16.10^{c} \pm 0.39$
15	$10.38^{d} \pm 0.20$

Activity is expressed as nanomoles CDNB conjugates min^{-1}mg $protein^{-1}$. Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

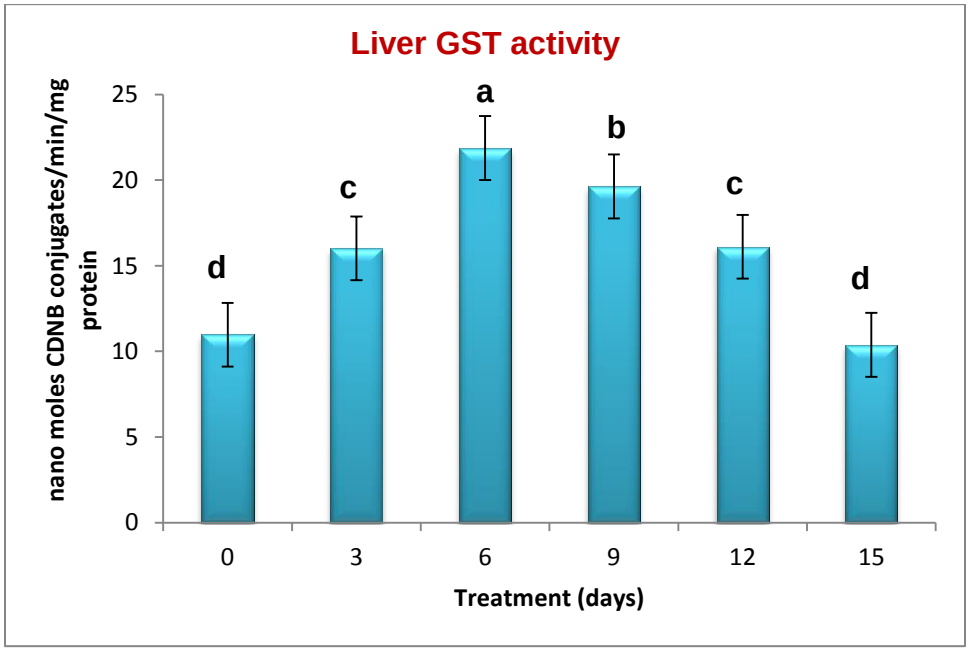


Fig. 58. GST activity in liver of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

4.5.6. Lactate dehydrogenase (LDH)

Lactate dehydrogenase activity of *Labeo rohita* juveniles was estimated at 0, 3, 6, 9, 12 and 15 days interval in liver and muscle after feeding with EMB @ 20 mg kg^{-1} body weight and the results are shown in table 15 and figure 59 & 60. The activity of the LDH was significantly different ($p<0.05$) among different sampling intervals. The LDH activity was highest at 6 days in both liver (21.45 ± 0.76) and muscle (17.24 ± 0.61).

Table. 15. Day wise change in LDH activity in liver and muscle of *L. rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

Treatment (days)	Muscle	Liver
0	$8.55^{d} \pm 0.62$	$9.30^{d} \pm 0.29$
3	$11.64^{c} \pm 0.72$	$12.68^{c} \pm 0.39$
6	$17.24^{a} \pm 0.61$	$21.45^{a} \pm 0.76$
9	$15.17^{b} \pm 0.58$	$15.90^{b} \pm 0.81$
12	$11.72^{c} \pm 0.31$	$11.56^{c} \pm 0.39$
15	$7.75^{d} \pm 0.75$	$8.92^{d} \pm 0.68$

Activity is expressed as micromoles mg $protein^{-1}min^{-1}$. Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

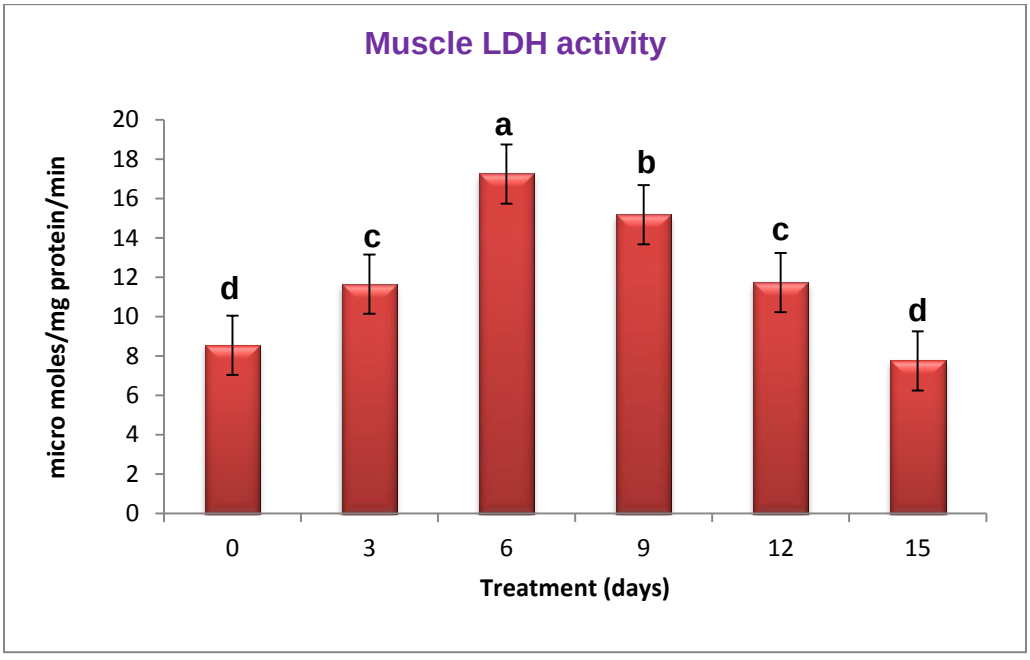


Fig. 59. LDH activity in muscle of *L. rohita,* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

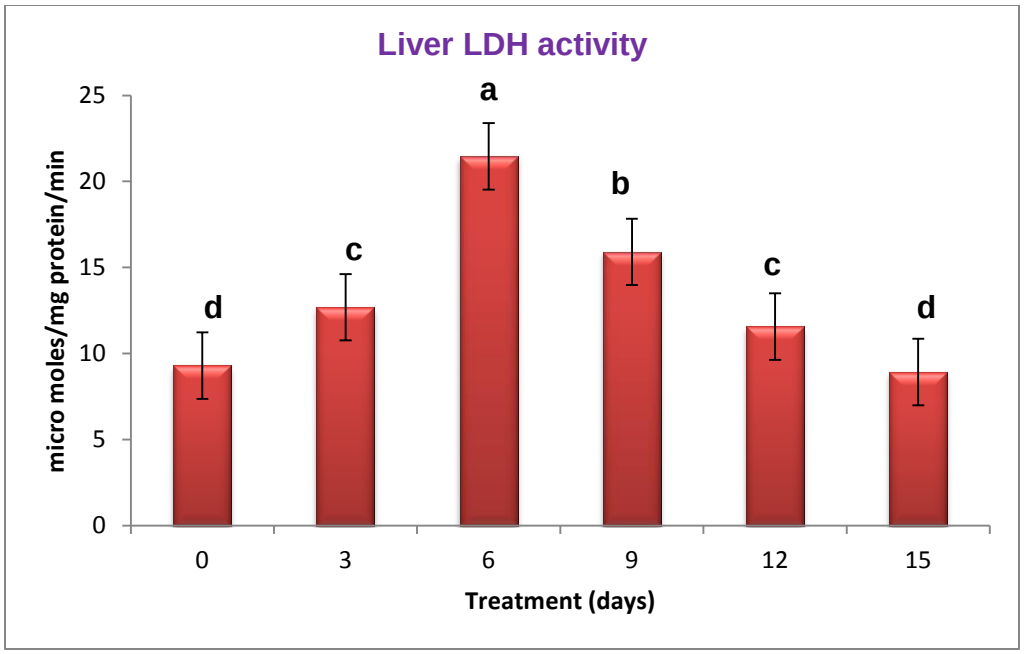


Fig. 60. Day wise change in LDH activity in liver of *L. rohita*, fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

4.5.7. Glutathione peroxidase (GPx)

The Glutathione peroxidase (GPx) activity of *Labeo rohita* juveniles was estimated at 0, 3, 6, 9, 12 and 15 days interval in the liver after post feeding EMB @ 20 mg kg^{-1} body weight and the results are shown in table 16 and figure 61. The activity of the GST enzyme found in different experimental days was significantly different ($p<0.05$). The hepatic GST activity was highest at 6 days post administration (3.54 ±0.02).

Table. 16. GPx activity in liver of *L. rohita,* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

Treatment (days)	Liver
0	1.75^{d} ± 0.11
3	2.92^{b} ± 0.08

6	$3.54^{a} \pm 0.02$
9	$2.70^{b} \pm 0.05$
12	$2.23^{c} \pm 0.10$
15	$1.50^{d} \pm 0.06$

Activity is expressed as oxidation of 1nmole of NADPH to $NADH^{+}$ min^{-1}mg $protein^{-1}$. Data (n=3) is represented the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

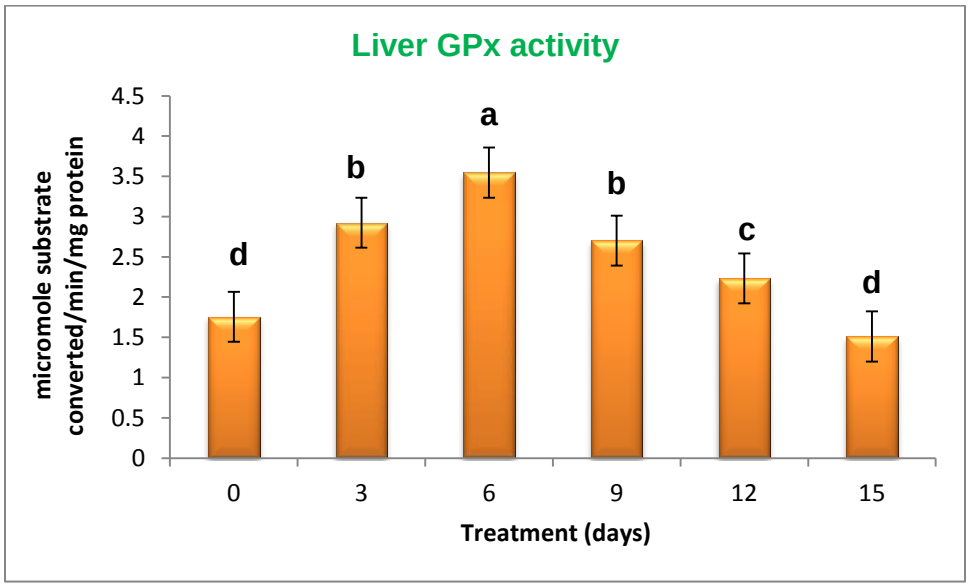


Fig. 61. GPx activity in liver of *Labeo rohita* fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

4.5.8. Alkaline phosphatase (ALP)

In the present study, the ALP activity was estimated at 0, 3, 6, 9, 12 and 15 days interval in the serum of *Labeo rohita* after post feeding EMB @ 20 mg kg^{-1} body weight and the results are shown in table 17 and figure 62. The ALP activity varied significantly among different treatments. The highest ALP activity was recorded on the 6-day post-feeding of EMB (14.54 ± 1.19).

Table.17. ALP activity in the serum of *L. rohita*, fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

Treatment (days)	Serum
0	$7.27^{c} \pm 0.33$
3	$10.00^{b} \pm 0.32$
6	$14.54^{a} \pm 1.19$
9	$10.43^{b} \pm 0.63$
12	$9.57^{b} \pm 0.64$
15	$7.07^{c} \pm 0.72$

Activity is expressed as nanomoles of PNP released min^{-1}mg $protein^{-1}$. Data (n=3) is represented as mean ± SE; Mean values under each column vary significantly ($p<0.05$).

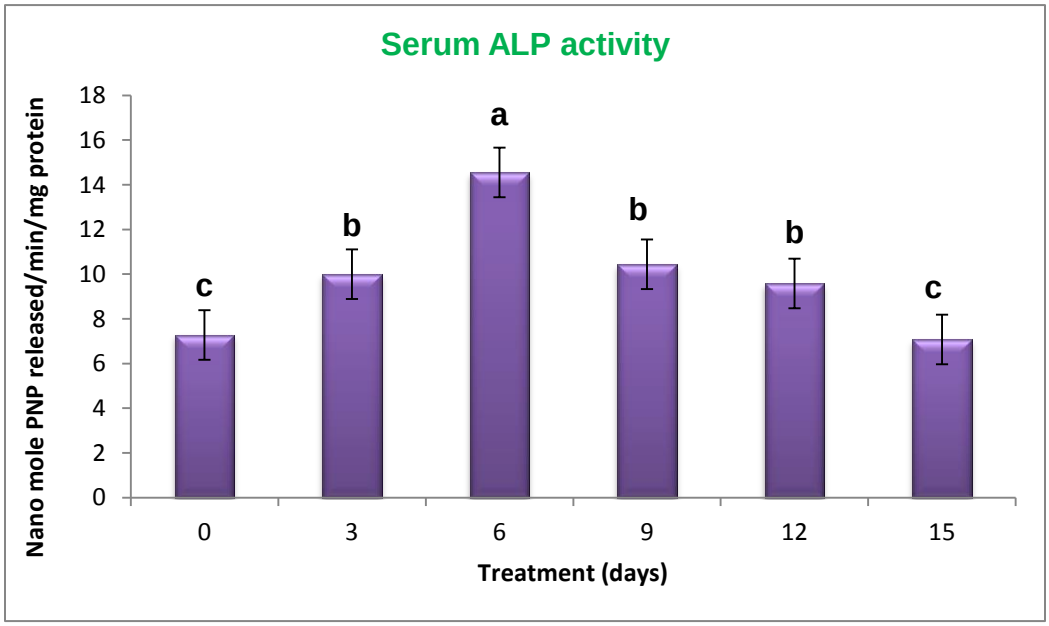


Fig. 62. Day wise change in ALP activity in serum of *L. rohita*, fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

4.6. EROD activity

4.6.1. EROD activity in liver of *Labeo rohita* post of EMB

The EROD activity in microsome fraction of liver fed with a single dose of EMB contained feed was assayed to study the induction of CYP4501A superfamily enzymes. The standard curve was plotted with resorufin at excitation and emission wavelengths of 535 nm and 585 nm respectively. EROD activity was found in the range of 11.53 ± 0.46 pmol μl^{-1} to 25.44 ± 0.51 pmol μl^{-1}, with the highest activity on the 6 day of post-feeding of EMB. The results are represented as table 18 and Figure 63 and 64.

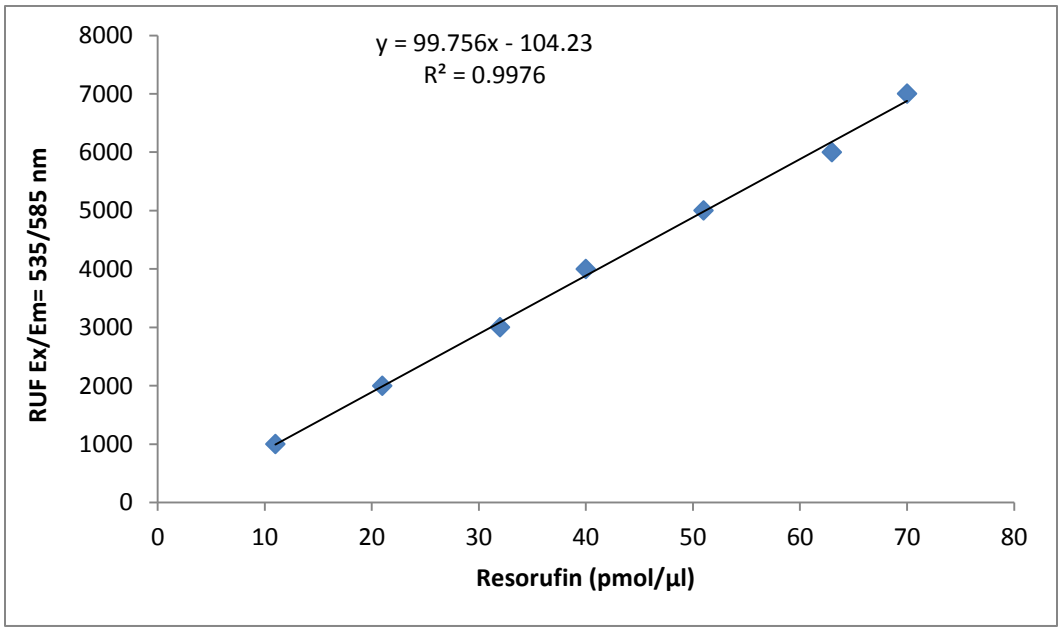


Fig. 63. Calibration curve of Resorufin

Table. 18. Day wise change in EROD activity in microsome fraction of liver of *L. rohita*, fed with a single dose EMB @ 20 mg kg^{-1} through the feed of.

Treatment (days)	Liver microsome
0	11.53^{e} ± 0.46
3	15.56^{c} ± 0.55

6	$25.44^{a} \pm 0.51$
9	$18.24^{b} \pm 0.59$
12	$16.09^{c} \pm 0.19$
15	$12.91^{d} \pm 0.18$

EROD activity expressed as unit pmol min^{-1}mg $protein^{-1}$. Each time point (n=3) represent the mean ± SE; Mean values under each column vary significantly ($p<0.05$).

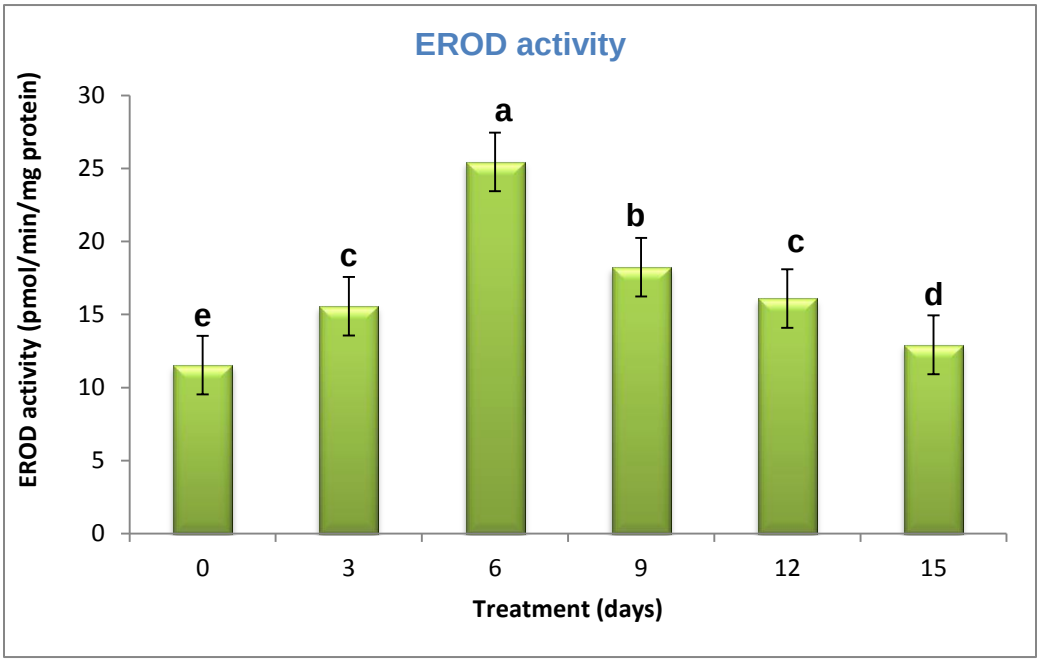


Fig. 64. Day wise change in EROD activity in microsome fraction of liver of *L. rohita*, fed with a single dose of EMB @ 20 mg kg^{-1} through the feed.

5. DISCUSSION

Aquaculture remains as one of the major export earning sector for many developing countries and has a significant impact on food security. The outbreak of infectious diseases is one of the major constraints in an aquaculture production resulting from mortality and reduced yield (Kumar *et al.,* 2015), which also affect fish trade. As such, there is no guideline prescribed infectious disease in fish, similarly in terrestrial animals and humans. Therefore, aquaculturists use same drugs approved for veterinary practice for treating infectious diseases in fish because there are no specific drugs developed specifically for fish (Santos and Ramos, 2016).

Several studies were carried out to check the efficacy of emamectin benzoate and its related compounds in *Gadus morhua* (Samuelsen, 2010; Hamre *et al.,* 2011),*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) *Dicentrarchus labrax* (Toksen *et al.,* 2006), *Anguilla rostrata* (Larrat *et al.,* 2010), *Oncorhynchus mykiss* (Hakalahti *et al.,* 2004; Roy *et al.,* 2006) for treating parasitic infection. There are no pharmacokinetic studies of EMB in the tropical fishes. Therefore, the present study was undertaken to understand the pharmacokinetics of emamectin benzoate and physio-metabolic responses in *Labeo rohita* administered with EMB through the feed. The result of the study provided a substantial support to determine an effective dose for *Labeo rohita,* which is an important prerequisite for the preparation of medicated feed.

5.1. Physico-chemical parameters of water

Physico-chemical parameters of waters such as temperature, pH, dissolved oxygen, free carbon dioxide, carbonate hardness, ammonia, nitrite-N, nitrate-N, were observed to be within the optimum range of requirements for fish culture.

Temperature plays an important role in regulating the metabolism of animals, so an optimum range of temperature is required for optimum metabolic activity, which in terms gives maximum yields. *Labeo rohita* can thrive well at a temperature range of 18.3^0C and 37.8^0C (Jhingran, 1991), which supports the range of temperature

28^0C to 30^0C was maintained during the entire experimental period of present study. The pH of the water in all the experimental groups was ranged from 7.8 to 8.1, which is within the acceptable range (6.7-8.6) as suggested by Andrew *et al.,* (1972).

The dissolved oxygen level in water varies with a large number of factors such as water temperature, metabolic rate, biomass density etc. The dissolved oxygen level in different experimental tanks was recorded to be within the range from 6.7 to 7.1 mg L^{-1} which are within the optimum range of 5.0-8.0 mg L^{-1} as suggested by Ali *et al.* (2000). From the above results, it is assumed that dissolved oxygen was optimum throughout the experimental period, through the provision of the aeration system. The carbon dioxide concentration was found to be negligible and hence no adverse effect was found on the survival and performance of the experimental animals. This might be due to low biomass per tank and daily water exchange during the experimental period.

The carbonate hardness was found to be in the range of 238-245 mg L^{-1} during the experimental period. Schaperclaus (1933) suggested water having a hardness of 250 mg L^{-1} or above as satisfactory for the growth of fish. Nitrite concentration was recorded in the range of less than of 0.05 to 0.1 mg L^{-1} which are well within the permissible range for pond aquaculture practices (Boyd and Tucker 1998). Nitrate-n level in a productive pond can be within 0.5 to 1 mg L^{-1} (Boyd and Tucker, 1998). In the present study, Nitrate-N was below the toxic level and hence it did not adversely affect the reared fishes.

5.2. Standardization of HPLC conditions

HPLC with UV- visible detector at 244 nm wavelength was used to quantify emamectin benzoate in plasma samples of *Labeo rohita*. All the chromatograms were obtained without any interference in a peak at the retention time of EMB. The retention time for emamectin benzoate was 5.7± 0.1 min. The calibration curve of emamectin benzoate was linear over the investigated concentration ranges of 0.05 µg ml^{-1} to 100 µg ml^{-1} and the linear regression equation of standard curve have shown satisfactory linearity with a coefficient of determination of 0.972, which is similar to the value reported by Cox *et al.,* (2009) for metronidazole.

5.3. Depletion profile of emamectin benzoate

Emamectin benzoate was administered at 10, 20, and 50 mg kg^{-1} of BW by intubation to find its effective therapeutic dose of EMB in *Labeo rohita*. The drug administered through intubation at the level of 10 mg kg^{-1} could not be detected in the plasma because the concentration was not sufficient to reach the blood. The highest concentration of EMB in the plasma detected after 1 h of post-administration at 20 mg kg^{-1} of BW and then reduced significantly. Fishes administered with a dose of 50 mg kg^{-1} of BW was showing higher plasma concentration even after 120 h compared to EMB at 20 mg kg^{-1} of BW.

The dose of 20 mg kg^{-1} was found to be sufficient to attach a desirable level of drug in the blood, which can elicit antiparasitic activity and eliminated within 120 h. Hence EMB at 20 mg kg^{-1} of BW was selected for medicated feed formulation. A feeding trial of medicated feed containing 0.2% EMB was carried out. A similar trend of intubation administration method was reported for feeding trial. The plasma concentration of EMB was lower in the case of fishes received a medicated feed in comparison to the EMB intubated fishes at the same dose. The total concentration of EMB in plasma of the fishes intubated and supplemented with medicated feed at 20 mg kg^{-1} of BW was 67.5%, and 18.17% respectively while absorption other avermectin derivatives in the gastrointestinal tract of human beings were only 5-10% (Reynolds and Martindale, 1996). This may be due to the fact that the agastric alimentary canal of rohu might have helped in the more efficient absorption from the intestine. Similarly, the plasma concentrations for derivatives of benzimidazole such as fenbendazole and mebendazole was 0.16% and 0.26% of fed amount respectively, which was less than the EMB concentration in plasma. This indicated that the effective absorption of EMB from GI tract was better than absorption of other anti-parasitic derivatives.

5.4. Residual concentration

The medicated feed was prepared by including EMB at 0.2% of BW and was administered to experimental fishes in a single dose. The parent residue of EMB in fish muscle was estimated at 0, 3, 6, 9, 12, and 15 days by standardized HPLC method.

The residue amount was detected maximum on 6 days of post administration. There was no residue was detected after 12 days of post administration whereas, in rainbow trout, the residue of EMB remained for 77 days (Roy *et al.,* 2006). This might be due to slow metabolism of the drug in the temperate climate. There is no parallel literature available regarding the residual limit of EMB in tropical fishes.

5.5. Enzyme assays

Lactate dehydrogenase activity in the liver was increased up to the 6 days of post-feeding and started to decrease afterward till 15 days. Similarly, activities of AST and ALT were also found higher on the 6 days (table 11 and 12). The higher activity of AST and ALT indicated the mobilization of aspartate and alanine which result in the keto acid utilization from the Krebs cycle to cope with the stress following drug intake. Similarly, Knox and Greengard (1965) reported that elevated level of transaminase activity during stress would lead to increased production of keto acid into TCA cycle thereby affecting oxidative metabolism. These studies have shown that transaminase activities increased during stress. These enzyme activities were at a normal level after the 15 days of post feeding, so it can be inferred that EMB oral administration @ 20 mg kg^{-1} BW had not caused metabolic stress in *Labeo rohita*. Similarly, Nautiyal (2014) reported the induction of ALT and AST activity in *Labeo rohita* fed with albendazole containing medicated feed.

Oxidative stress is well indicated by antioxidant enzymes such as SOD, CAT, GST, GPx etc. The activity of catalase, SOD, Glutathione S-transferase(GST) was increased after post-administration of EMB. The results showed that the drug has potentiated antioxidant defenses in the body of the animal as reported by Lushchak (2001) for metronidazole.

Superoxide dismutase (SOD) serves as a biomarker of oxidative stress and antioxidant status of organisms (Kohen and Nyska, 2002). It is an important defense in all the cells exposed to reactive oxygen species. SOD is the most important antioxidant enzyme acts against toxic effects of free radicals and oxygen metabolism (Costantini, 2008). Emamectin benzoate renders a remarkable antiprotozoal

(ectoparasitic) activity and widely used in the treatment and control of various parasites The activity of SOD was studied in both liver and gill and highest SOD activity was found on the 6 days in the liver and gill. After the 6 days, the SOD activity was similar to control in other time intervals. It was revealed that the SOD activity was induced in both liver and gill when the fishes are administered with emamectin benzoate containing feed.

Catalase is the primary cellular enzymatic defense against H_2O_2, which convert it into H_2O and O_2 and is critical for the process of scavenging free radicals (Dorval *et al.,* 2003). In the present study, catalase activity was found highest in liver and gill on the 6 days of post-feeding and was found lowest in both control and 15 days post feeding. The result is an agreement by Han *et al.,* (2003), who reported that elevated activities of SOD and catalase in common carp (*Cyprinus carpio*) when exposed to metronidazole.

Glutathione S-transferase (GST) is an important enzyme for the detoxification of potentially harmful substances, including reactive oxygen species, lipid peroxidation products and electrophilic compounds (Torres-Rivera *et al.,* 2008). Gonzalez *et al.*, (2008) studied the GST activity in relation to ABZ in fish but did not find a regular trend. GST play a critical role in mitigating oxidative stress in all life forms and GST activity also has been widely used as a biomarker to detect stress. In the present study, GST activity was found highest in liver on 6 day post administration.

Glutathione peroxidase (GPx) is a selenium (Se) containing enzyme catalyzing the reduction of hydrogen peroxide or lipid peroxidase by reduced glutathione (Cohen and Hochstein, 1963; Christopherson, 1969). GPx activity was highest in 6 day post feeding and relapsed to normal levels within 15 days.

5.6. EROD activity in the liver of *Labeo rohita* fed with EMB

CYP1A is considered one of the best marker of detoxification of xenobiotics in the liver of human beings, animals and aquatic organisms (Dar *et al.,* 2017; Stegeman and Hahn, 1994). Measurement of CYP induction in response to a drug is a fundamental aspect of assessing drug interactions and evaluating drug safety

and efficacy. EROD activity has been used as an indicator of CYP1A induction in fish (Whyte *et al.,* 2000). In the present study, the EROD activity was estimated in liver microsomal bodies after administration of the EMB in feed @ 20 mg kg^{-1} of body weight in fishes and found 1.9 to 2.5 folds increase in activity in comparison to control. Similarly, Gonzalez *et al.,* (2008) reported a 2.2 to 2.6 fold increase in the activity of CYP1A in channel catfish fed with albendazole. The present study showed highest CYP1A activity on the 6 day of post administration. This indicated the efficient detoxification of emamectin benzoate through cytochrome P450 dependent (phase I) subsequently by conjugation (phase II) in fishes.

The result obtained from the study showed that emamectin benzoate containing feed regarded as a safe feed to IMC, which is to be used for curing the ectoparasitic infection. The drug residue as well as metabolic stress due to EMB was reflected in tissues after 6 days of administration and later reduced to normal and eliminated by the 12 day. Hence, the study recommends the single dose of EMB at 20 mg kg^{-1} of BW can effectively reach into the blood and tissues of *L. rohita* without creating any adverse effect in fish and fish consumers.

6. SUMMARY

In the present study, one hundred sixty-two healthy juveniles of *Labeo rohita* were randomly distributed in 27 experimental tanks with six fishes in each tank. Among them 27 tanks received three doses of EMB (9 tanks for one dose, like 10 mg kg^{-1}, 20 mg kg^{-1}, and 50 mg kg^{-1}), one tank was kept as control and does not receive any treatment and samples were collected on 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 92, 120, 144 , and 168 h. For biochemical analysis, 21 fishes were randomly distributed in 3 tanks. Seven fishes were kept in each tank. The fishes in 3 tanks of total 21 received a single dose of EMB 20 mg kg^{-1} through feed method. All the fishes were fed with a purified diet of crude protein 32.8% and lipid 6% throughout the experimental period and fishes were not fed one were prior to EMB administration. Sampling was done on, the 0, 3, 6, 9, 12, and 15 days of post administration. At each sampling time, thee fishes were sacrificed. One tank received no EMB and treated as control. At each time point, from each tank blood and tissue sample were collected from 3 fishes. The collected blood samples were processed and subjected to HPLC analysis for detection of EMB level in the plasma. All water quality parameters were in the optimum range throughout the experimental period.

HPLC with UV-visible detector, reverse phase analytical column, isocratically at a flow rate of 1 ml/min and mobile phase consisted of 0.1% orthophosphoric acid and acetonitrile at 60:40 (0.1% H_3PO_4: ACN) were used to detect EMB metabolism in plasma of rohu. EMB was detected up to 144 h post dose of emamectin benzoate suspension and highest concentrations were detected at the 1 h time point.

For biochemical analysis, twenty-one fishes were randomly distributed in three tanks @ 7 fishes in each tank, receiving dose @ 20 mg kg^{-1} body weight of EMB respectively and a tank was kept as a control receiving no treatment. Experimental animals were sacrificed in triplicates and collected liver, gill, muscle, and intestine. Samples were collected on 3, 6, 9, 12 and 15 days of post-feeding. Metabolic enzymes

(AST, ALT) oxidative stress enzymes (SOD, catalase, and GST) and EROD activity were studied to evaluate physio-metabolic responses.

Pharmacokinetics of EMB in Rohu suggested that metabolism of this drug in rohu was slow so it tends to be for a longer time in gastrointestinal tract and can exert its anthelminthic action for a longer time. Whereas, metabolism of EMB is very slow in fish as its concentration remains higher up to end of the experimental period and exerts a harmful effect as compared to its effect on the ectoparasites. In the present study, the medicated feed was prepared for fishes with emamectin benzoate with gel coating around the molecule in order to prevent the leaching of the drug and its concentration was enough to reach the drug into the blood indicating its absorptive efficacy. The drug was cleared within 120 h from the system indicating its safety in *Labeo rohita.*

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APPENDIX

µg	Microgram
A	Absorbance
ABZ	Albendazole
ACN	Acetonitrile
ALP	Alkaline Phosphatase
ANOVA	Analysis Of Variance
AUC	Area Under Curve
BHT.	Butylated Hydroxytoluene
BSA	Bovine Serum Albumin
BSA	Bovine Serum Albumin
BW	Body Weight
C^{14}	Carbon 14
CAT	Catalase
C_{max}	Maximum Serum Concentration A Drug Can Achieves
CMC	Carboxy Methyl Cellulose
CP	Crude Protein
CYP 450 1A	Cytochrome P450, Member A1
DE	Digestible Energy
DO	Dissolved Oxygen
EDTA	Ethylenediaminetetraacetic Acid
EE	Ether Extracts
EMB	Emamectin Benzoate
EMB	Emamectin Benzoate
EROD	Ethoxy-O- Resorufin
FAO	Food And Agriculture Organization
FRP	Fibre-Reinforced Plastic
g	Gram

GABA	Gamma-Aminobutyric Acid
GDP	Gross Domestic Product
GI	Gastro Intestinal Tract
GOT/ AST	Glutamic Oxaloacetic Transaminase/ Aspartate Transaminase
GPT/ALT	Glutamate-Pyruvate Transaminase/ Alanine Transaminase
GPx	Glutathione Peroxidase
GSH	Glutathione
GSSG	Oxidized Glutathione
GST	Glutathione S-Transferase
h	Hour
H_2O_2	Hydrogen Peroxide
H3PO4	Phosphoric Acid
HPLC	High-Performance Liquid Chromatography
HPLC	High Performance Liquid Chromatography
IMC	Indian Major Carp
KCl	Potassium Chloride
kg	Kilogram
KH2PO4	Monopotassium Phosphate
KMnO4	Potassium Permanganate
L	Liter
LDH	Lactate Dehydrogenase
mg	Milligram
ml	Milliliter
n mole	Nano Mole
Na_2HPO_4	Disodium Phosphate
NaCl	Sodium Chloride
NaCl	Sodium Chloride
$NADP^+$	Nicotinamide Adenine Dinucleotide

	Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium Hydroxide
NFE	Nitrogen Free Extract
OD	Optical Density
pH	Potential Of Hydrogen
pmole	picomole
PNP	P-nitrophenol
R^2	Regression Co-Efficient
RPM	Revaluation Per Minute
RT	Retention Time
SOD	Superoxide Dismutase
SPSS	Statistical Package for the Scoial Sciences
TC	Total Carbohydrate
T_{max}	The Time At Which C_{max} Achieves
UV	Ultra Violate

LIST OF FIGURES

LIST OF TABLES