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Electrophoretic studies of tissue specific inhibition of esterases in different tissues (Intestine, Muscle, Brain) of fresh water Fish *Channa punctatus* exposed to enzyme inhibitors Paraxon, Eserine, Parachloro Mercuric Benzoate

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ABSTRACT

The current research was under taken to discover the electrophoretic studies of tissue specific inhibition of esterases in different tissues (intestine, muscle, brain) of *Channa punctatus* exposed to enzyme inhibitors i.e. Paraxon, Eserine (Physostigmine), Parachloro Mercuric Benzoate (pCMB). The inhibition studies of Esterases were conducted as per the standardized procedures. The results revealed that Intestine tissue E-1 are CHsp esterases which are inhibited by all the three inhibitors. E-2 are Carboxyl Esterases (CE) which are inhibited by paraoxon only. While E-3 are Ese-Esterases which are inhibited by eserine only. Muscle tissue E-1 are CHsp, E-2 are enzymes resistant to inhibitors (ER)esterases, which are not inhibited by all inhibitors, E-3 are Ese-esterases which are inhibited by eserene only. Brain tissue E-1 and E-2 are Carboxyl Esterases (CE) which are inhibited by paraoxon only, E-3 are Cholinesterases (ChE) which are inhibited by both paraoxon and eserine.

Keywords: *Channa punctatus*, electrophoretic studies, intestine, muscle, brain, tissue specific inhibition, Esterases, Paraxon, Eserine, pCMB,

INTRODUCTION

The fish is rich in essential amino acids and can complement or fulfill the overall protein quality that is required in mixed diet (Pathak *et al*., 2015; Louka, *et al*., 2004; Dempson, *et al*., 2004). Fish can be used as an excellent model for monitoring environmental contamination affected by water pollution (Scott *et al*., 2004; Shinde et al., 2007, Esterases are multifunctional, lipid hydrolyzing enzymes occurring in multiple forms and capable of separation by electrophoresis (King 1974).

Esterase enzyme may be of particular importance because fish utilize lipid/fat as their main nutritional source rather than carbohydrates and protein especially during the later developmental stages (Baglole et al., 1998).

The use of biomarkers in monitoring pollution and hence, in pollution environmental risk assessment, is an important tool to evaluate early warning signs of pollution for the effect measurement in organisms (McCarthy and Shugart, 1990). These enzymes appear to be playing a critical role in offering resistance to insecticides (Karunaratne et al, 1999) and used as a bioindicator to measure the toxic potency of pesticide residues usually applied in agriculture. The residual effect of pesticide in aquaculture specifically in fish which in-turn cause death of fish (Debnath 1978), (Sahib et al., 1980), (Begum et al., 2008). Loevenhert (1906) introduced the term esterases and lipases for the enzymes that catalyse the formation and breakdown of carboxylic acid esters and alcohols and glycerol. Electrophoretic pattern of esterases of different tissues show species specific variation, it could be successfully used for the identification of fish species" (Shengming, et al., 1988). "Electrophoretic studies were done extensively on the different tissues of various animals from which it reveals that the enzymes exist in multimolecular forms and perform various functions" Markert and Moller, 1959). The present research is done on the Electrophoretic banding pattern of Esterase Isozymes in different tissues i.e. intestine, muscle, brain of fresh water fish *Channa punctatus (Bloch) is* on the basis of the electrophoretic motilities of individual zones and inhibitor sensitivity of individual zones to three inhibitors.

i. Paraoxon (an organophosphate),
ii. Eserine (Physostigmine) (a carbamate) and
iii. Parachloromercuric benzoate (pCMB - A thiol active compound).

Paraoxon (an organophosphate), Eserine (a carbamate) and pCMB (a thiol group inhibitor). Paraoxon was found to be as effective in inhibiting the carboxylesterases" Metcalf et al., 1972, Reddy et al., 1988) and "Eserine was used as a criterion for detecting cholinesterases which were found sensitive to both organophosphates and carbamates" (Holmes et al., 1968; Lakshmipathi and Reddy, 1989, 1990, Lakshmipathi, 1994). "Arylesterases were inhibited by pCMB alone. The enzyme which exhibited mixed inhibition were classified as Esdpesterases (inhibited by Paraoxon and pCMB), CHsp esterases (inhibited by all the three inhibitors) and Ese esterases (which were inhibited only by eserine), (Haritos and Salamastrakis, 1982). Cholinesterases (ChEs) namely acetylcholine-esterase - AChE – (EC3.1.1.7), regulate nerve impulse transmission by hydrolysis of the neurotransmitter acetylcholine in vertebrates and invertebrates (Thompson and Walker, 1992). Pesticides may cause an increase in the production of reactive oxygen species (ROS) in aquatic organisms which may lead to impairment of many cellular functions (Uner *et al.,* 2006; Monterio *et al.,* 2009; Modesto and Martinez 2010; De Menezes *et al.,* 2011). The deleterious effect of free radicals can be prevented or counterbalanced by antioxidant systems (Lushchak, 2011; Singh *et al.,* 2011)

The Eseterase Isozymes are classified as follows

1. Carboxylesterases (CE): These esterases were sensitive to inhibition by the organophosphate but were not affected by physostigmine or pCMB.
2. Arylesterases (ArE): They were sensitive to inhibition by sulphydryl Agent pCMB and were not affected by paraoxon or physostigmine.
3. Cholinesterases (ChE): Enzymes, which were inhibited by paraoxon and physostigmine.
4. ER Esterases: Enzyme which were not affected by any of the three inhibitors used.
5. EsdpEsterases: Enzymes, which were inhibited by pCMB and paraoxon.
6. Ese Esterases: Enzymes, which were inhibited by physostigmine alone.
7. CHsp Esterases: Enzymes, which were inhibited by paraoxon, physostigmine and pCMB (Quave et al., 2008;

Shahjahan et al. (2008) Callaghan et al. (1994) Manju *et al.* (2017) submitted Malathion dimethoate and Chlorpyrifos induce inhibitory effect on AChE activity, while maximum inhibition was induced by Malathion. The esterases show specific banding patterns in different tissues of various species it could be used for the identification of fish species (Stordeur, 1976 and Shengming *et al.,*1988). Effect of Parathion on *Channa punctatus* studied by (Rajaiah and Venkaiah, 2007) and reported that parathion affected the esterase enzymes in fish. This characteristic makes them good biochemical markers (Staykova, 2008).

MATERIAL AND METHODS

The adult fishes (weighed about 50-70g) were collected from ponds (tanks) located within the radius of 60kms from Kakatiya University campus by netting with the help of local fishermen. They were immediately brought to the laboratory in plastic buckets and acclimatized to laboratory conditions for about a week in aquaria. They were fed on natural plankton collected from their natural habitats. Fishes were immobilized and the tissues were dissected out from the animals. Five tissues were selected for the study i.e. intestine, muscle and brain. The tissues from (adult fishes) six individuals were collected from ice jacketed containers. After collecting the tissues blotted to free from blood clots and other adherent tissues and weighed to the nearest milligram and were homogenized in 0.01N Tris. HCL buffer (Ph =7.5) containing 0.9% of Nacl. The concentration of tissue homogenates varied from tissue to tissue i) Gill 10% ii) Liver 10% iii) Intestine 10%. The homogenates were centri-fuged at 2000 rpm for 10 min on a clinical centrifuge at room temperature. The supernatant was mixed with equal volumes of 20% sucrose solution containing 0.05% bromophenol blue as the tracking dye. An aliquot of 0.1 ml of this mixture was used for loading the sample on to the separating gel for separation of esterase patterns. (Holmes and Masters, 1967, Reddy and Lakshmipathi, 1988). Esterase patterns were separated on thin layer 1.5mm (thickness) poly-acrylamide gels (7.5%). The gel mixture was prepared according to Clark- 1959. Gelling was allowed to 45 min, after loading the samples on the gels, the samples were laid with electrode buffer on gel plates were connected to the electrophoretic tank. Tris (0.05M), glycine (0.38M) buffer (PH=8.3) was used as the electrode buffer. A constant current of 50 volts for the first 15 min followed by 150 volts for the rest of the run was supplied during the electrophoresis. The electrophoretic run was terminated when the tracking dye migrated to the distance of 5cm from the origin. Esterases were visualized on the gels by adapting the staining procedures of (Raju and Venkaiah 2013; Rao et al., 2018; Shankar et al., 2019). They were stained for esterase activity with α- naphthyl acetate as substrate. (Reddy and Lakshmipathi 1988).

After visualizing the electrophoretic banding patterns of Esterase isozymes in the different tissue i.e. intestine, muscle, brain the control tissue esterases were exposed to enzyme inhibitors i.e. Paraxon (an Organophosphate), Parachloromercuric benzoate (pCMB - A thiol active compound). and Eserine (Physostigmine, a carbamate) then the Electrophoretic banding patterns of Esterase isozymes were visualized and then the enzyme inhibition was identified and enzyme Inhibitor studies were conducted as per the standardized procedures *in vitro* Venkaiah and Lakshmipathi (2006). Then the esterase isoenzymes were classified based on inhibitor study.

RESULTS

Intestine

Intestine tissue showed 03 Esterase isoenzymes. Est-1 with Rm value 0.6± 0.05, Est-2 with Rm value 0.4± 0.05 and Est-3 with Rm value 0.3± 0.05. Est-1 was inhibited by Paraoxon, Eserine and pCMB. Hence Est-1 was classified as CHsp Est-2 were inhibited by only Paraoxon, not inhibited by Eserine and pCMB. Hence Est-2 was classified as Carboxylesterases (CE). Est-3 was inhibited by Eserine only (Ese esterase), not inhibited by Paraoxon and pCMB. (Fig 1 and Table 1).

Muscle

Muscle tissue showed 03 esterase isoenzymes. Est-1 with Rm value 0.6± 0.05, Est-2 with Rm value 0.4± 0.05 and Est-3 with Rm value 0.3± 0.05. Est-1 was inhibited by Paraoxon, Eserine and pCMB. Hence Est-1 was classified as CHsp. Est-2 was not inhibited by Paraoxon, Eserine and Pcmb. Hence Est-2 was classified as E.R esterase. Est-3 was inhibited by Eserine only. Hence Est-3 was classified as Ese esterase (Fig 2 and Table 2).

Table 1: Tissue specific Inhibition of esterase in Intestine tissue of *Channa Punctatus* and classification of enzymes

Intestine	Est-1	Est-2	Est-3
Control	+++	+++	++
Paraoxon	+	+	-
Eserene	+	-	+
pCMB	+	-	-
Classification	CHsp	CE	Ese-Est

CHsp= enzymes inhibited by paraoxon, eserene, pCMB; CE= Enzymes inhibited by paraoxon only; Ee-est= enzymes inhibited by eserene only.

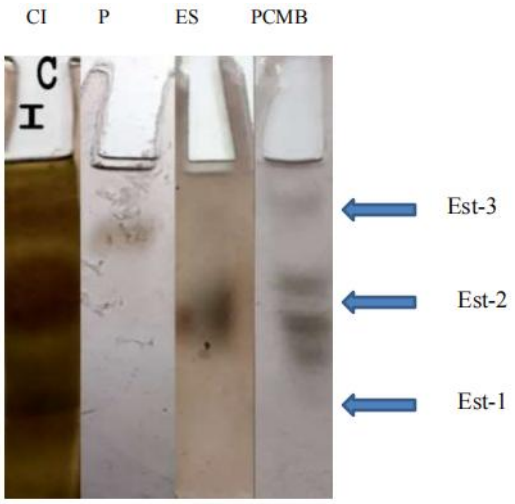

Figure 1: Electrophoretic patterns of esterases inhibition in intestine tissue of *Channa Punctatus*
CI = CONTROL INTESTINE; P= IN THE PRESENCE OF PARAOXON; Ese= THE PRESENCE OF ESERINE; pCMB= IN THE PRESENCE OF pCMB

Table 2: Tissue specific Inhibition of esterase in muscle tissue of *Channa Punctatus* and classification of enzymes

Muscle	Est-1	Est-2	Est-3
Control	+++	+++	+++
Paraoxon	+	-	-
Eserene	+	-	+
pCMB	+	-	-
Classification	CHsp	ER Est	Ese-Est

CHsp= Esterases inhibited by all inhibitors : ER est= Esterases resistant to inhibitors: Ese-est= Enzymes ihibited by eserene only

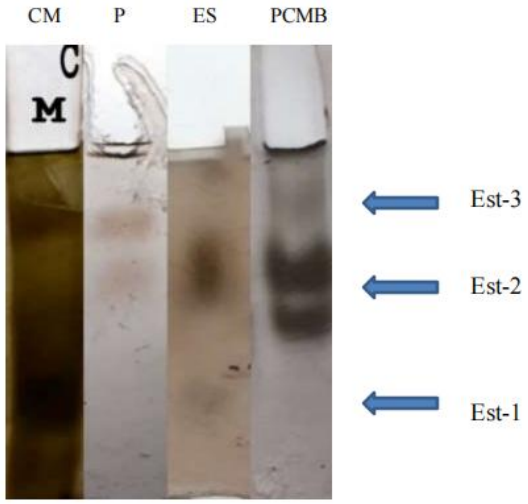

Figure 2:Electrophoretic patterns of esterases inhibition in muscle tissue of *Channa Punctatus*
CM = CONTROL MUSCLE; P= IN THE PRESENCE OF PARAOXON; Ese= IN THE PRESENCE OF ESERINE; pCMB= IN THE PRESENCE OF pCMB

Table 3:Tissue specific Inhibition of esterase in brain tissue of *Channa Punctatus* and classification of enzymes

Brain	Est-1	Est-2	Est-3
Control	+++	+++	+++
Paraoxon	+	+	+
Eserene	-	-	+
pCMB	-	-	-
Classification	CE	CE	ChE

CE= Enzymes inhibited by paraoxon only; ChE= enzymes inhibited by paraoxon and eserene

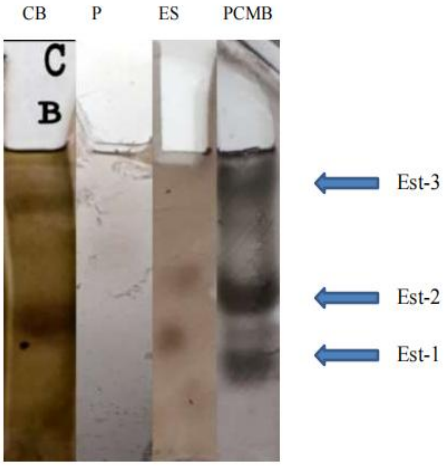

Figure 3:Electrophoretic patterns of esterases inhibition in brain tissue of *Channa Punctatus*
CB = CONTROL BRAIN; P= IN THE PRESENCE OF PARAOXON; Ese= THE PRESENCE OF ESERINE; pCMB= IN THE PRESENCE OF pCMB

Brain

Brain tissue showed 03 Esterase isoenzymes. Est-1 with Rm value 0.6± 0.05, Est-2 with Rm value 0.4± 0.05 and Est-3 with Rm value 0.3± 0.05. Est-1 and Est-2 were inhibited by Paraoxon only, not inhibited by Eserine and pCMB. Hence Est-1 and E-2 was classified as Carboxylesterases (CE). Est-3 was inhibited by both Paraoxon and Eserine, not inhibited by Pcmb. Hence Est-3 was classified as Cholinesterases (ChE) (Fig 3 and Table 3).

DISCUSSION

The present research study Tissue specific inhibition of Esterases in different tissues i.e. gill, liver, intestine, muscle and brain of fresh water fish Channa punctatus through Native- gel electrophoresis in the presence of some enzyme inhibitors i.e. paraoxon, Eserine and pCMB showed trajectory to the classification of the Esterases. Est-1 in intestine and muscle tissue are classified as CHsp which are inhibited by paraoxon, Eserine and pCMB and brain Est-1 was classified as Carboxylesterases(CE) which are inhibited by only Paraoxon. Est-2 in intestine and brain are carboxylesterases (CE) which are inhibited by paraoxon only and Est-2 of muscle are classified as ER-esterases, which are not inhibited by all the three enzyme inhibitors. Est-3 of intestine and muscle are inhibited by eserene only hence classified as Ese-esterases while E-3 in brain was classified as ChE , Choline esteases, which are inhibited both paraoxon and eserene. The current study reveals that high concentration of Caroxyl esterases were discovered in E-2 of intestine, E-1 and E-2 of brain. High concentration of CHsp were exposed in E-1 of intestine and muscle. And high concentration of E-3 in intestine and muscle were Ese-est.

Esterases play a significant role in the xenobiotic metabolism and hydrolyse or detoxify several foreign chemicals entering into the cells (Isabela Reis Montella *et al*., 2012). Carboxylesterases are chief conduits of detoxification in fishes (AI-Ghiar *et al.,* 2000). The alterations in the enzymatic parameters can be effectively used as potential biomarkers for monitoring of the Organophosphorous pesticides in the aquatic environment (Abhijith *et al.,* 2016). Esterase isoenzyme study in Electrophoresis was extensively studied by (Venkateswara Rao et al.,2023a, 2023b; 2023c, Venkateswara Rao et al.,2022). Our results are in consonance with (Bheem Rao et al., 2024, Rajaiah et al., 2010, Ch.Shankar et al., Venkaiah et al., 2006).

Kuster and Altenburger (2006) reported the comparison of Cholin and Carboxylase enzyme inhibition and visible effects in the Zebra fish in Gel Electrophoresis. (Shao -Nan Li and De Fang Fan, 1996) Reported the activity of Esterases from Different Tissues of fresh water fish and their responses of their Isoenzymes to Inhibitors in Gel Electrophoresis.

Lakshmipathi and Reddy (1989) discovered the Esterases in brain tissue of Vertebrates in Gel Electrophoresis. Tissue esterase patterns of muscle and brain of channiformes and perciformes fishes was studied in Gel Electrophoresis by Rajaiah et al., 2010. Distribution of six classes of esterases; viz. carboxyl, acetyl, aryl, choline, Esdp and ER-esterases in the two

tissues of the four species is reported based on the substrate sensitivity and inhibitor sensitivity (Lakshmipathi et al., 1989). Esterase patterns were discovered in Gel Electrophoresis in the three tissues viz skeletal muscle, brain and liver tissue of Amblypharyngodon mola are discussed. Physostigmine, pCMB, paraoxon and DFP are used to classify the esterases into different categories (Reddy and Lakshmipathi, 1988).

CONCLUSIONS

Our current research Esterase inhibition study in different tissues (Gill, Liver, Intestine) of fresh water Fish Channa punctatus through Gel Electrophoresis (Native gel electrophoresis) showed differential banding pattern of esterases which indicates the species specificity of the enzymes. Isoenzyme analysis has been used to estimate the genetic variation between different population of fish. Esterases are most widely used for species identification for both vertebrate and invertebrate, they are also used for embryological studies for the differentiation of specific tissue. Esterases are used as marker enzymes in biochemistry, cytochemistry and also in evolution. These are also used in bioremidiation, biodiesel production, insecticide detoxification and in population genetics. In our present research we found a wide variation in the esterase isozyme banding pattern could be very much useful for the development of genetic molecular markers.

Competing Interests
Authors have declared that no competing interests exist.

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