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Esterase Inhibition Study in Different Tissues (Intestine, Muscle and Brain) of Fresh Water Cat Fish *Heteropneustesfossilis* through Polyacrylamide Gel Electrophoresis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Esterase polymorphism was studied in different tissues of stinging catfish (*Heteropneustesfossilis)* viz; intestine, muscle and brain. Intestine showed ER esterases(Esterases resistant to inhibitors) and CE esterases (Carboxylesterases), muscle showed ChE (Cholinesterases) and CHspesterases (Enzymes which were inhibited by Paraoxon, Eserine, and pCMB) and brain showed ChE(Cholinesterases) and Esdp(Esterases inhibited by Eserine alone) esterases. The present study was carried out to find out tissue specific esterase banding patterns in different tissues of *H.fossilis*. Electrophoretic banding patterns of esterase of different tissues showed species specific variation which could be successfully used for identification of fish species. Electrophoretic pattern of esterases of different tissues show species specific variation, it could be successfully used for the identification of fish species

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1. INTRODUCTION

"Electrophoretic banding patterns of esterase of different tissues showed species specific variation which could be successfully used for identification of fish species. Electrophoretic pattern of esterases of different tissues show species specific variation, it could be successfully used for the identification of fish species" [1]. Tissue specific proteins and enzymes are of immense importance in recognizing species and establishing their taxonomic relationship in number of animal groups [2]. "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" [3].

Comparisons were made between the tissues of the same fish species and between the homologous tissues of different species on the basis of the electrophoretic motilities of individual zones and inhibitor sensitivity of individual zones to three inhibitors:

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

"Esterase patterns observed in the present study were classified on the basis of their sensitivity to three inhibitors viz., Paraoxon (an organophosphate), Eserine (a carbamate) and pCMB (a thiol group inhibitor). Paraoxon was found to be as effective in inhibiting the carboxylesterases" [4,5] and "Eserine was used as a criterion for detecting cholinesterases which were found sensitive to both organophosphates and carbamates" [6,7,8,9]. "Arylesterases were inhibited by pCMB alone. The enzyme which exhibited mixed inhibition were classified as Esdpesterases (inhibited by Paraoxon and pCMB), CHspesterases (inhibited by all the three inhibitors) and Ese esterases (which were inhibited only by eserine)" [7,8] (Haritos and Salamastrakis, 1982).

"The high region and spacio specificity of these enzymes has applications in the Kinetic resolution of optical isomers for synthesis of optically pure substances in pharmaceutical and chemical industries" [10] (Alam et al., 2015). "Their ability was to catalyze a variety of esterase without the aid of cofactors is an additional advantage" [10]. "Esterases play a vital role in the metamorphosis of insects" (Yu et al., 2009). The present study was aimed to investigate the comparative tissue of esterase polymorphism of selected prawns

Esterases are the enzymes which hydrolyze the esters of carboxylic acid. Lovenhart (1906), recognized two categories of such enzymes. Enzymes, which hydrolyze the esters of short chain (C2- C4) fatty acids were recognized as esterases, while those which hydrolyse the long chain fatty acid esters (> C8) were identified as lipases [11]. A great advance in the study of esterases was made when organophosphates were recognized as inhibitors of these enzymes (Webb, 1948). Aldrige [12] distinguished the enzymes into two 'A' and 'B' esterases, by using the organophosphate inhibitors E-600 (Diethyl- para nitro phenyl phosphate). 'B' esterases were reported to be sensitive to the compound where as the 'A' esterases hydrolyzed it. A third type of enzyme, 'C' esterases [13], were shown to be neither sensitive to organophosphates nor were capable of hydrolyzing them. Low concentrations of pCMB (Parachloromercuribenzoate) slightly activated these enzymes. The present study was carried out to find out tissue specific esterase banding patterns in different tissues of *H. fossilis*. [14]. The developmental multiplicity and isoenzyme status of cavian esterases. [6]. Electrophoretic differences of esterases isozymes from the surface mucous of Oreochromis fishes [15]. Esterases in amblypharyngodon mola [5]. Simplified "Disc" (Polyacrylamide Gel Electrophoresis [16]. Growth and reproduction of the slug Limax valentianus Férussac in experimental conditions. Journal of Molluscan Studies [17]. Overview of carboxylesterases and their role in the metabolism of insecticides [18]. A review on hydrolytic enzymes in the treatment of waste water with high oil and great content [19]. Analysis of AchE and LDH in mollusc, Lamellidens marginalis after exposure to chloropyrifos [20]. The ecology of shell shape difference in chirally dimorphic snails [21]. Esterase Variability in Different Tissues of Flying Frog Rhaco Phorus Lateralis) of Indian, using Polyacrylamide Gel electrophoresis [22]. Geographic phenetic variation in the golden apple snail, Pomacea canaliculata (Ampullariidae) based on geometric approaches to morphometrics [23].

2. MATERIALS AND METHODS

The fresh water cat fish *H. fossilis*were collected from local fresh water tanks within the radius of 15km from the laboratory by netting with the help of local fisher man. The fishes having an average length of 15 ± 1cm and weighed about 50±5gm were brought to the laboratory and transferred in to a plastic buckets (30X30X60cm) and disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection). The fishes were acclimatized for about 10 to 15 days prior to experimentation. They were regularly feed with commercial fish food and the medium (tap water) was changed daily to remove feaces and food remnants. The healthy fishes were grouped into five batches containing six each and were exposed to different concentrations of organophosphate methyl parathion at different time intervals to calculate the medium lethal concentration less value using probit analysis method

The scheme of classification employed in the study is as hereunder:

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.

CHspEsterases: Enzymes, which were inhibited by paraoxon, physostigmine and pCMB [24-27].

3. RESULTS

Esterase polymorphism were studied in different tissues of *Heteropneustesfossilis*three tissues were studied viz; intestine, muscle and brain

Intestine: Intestine showed four esterase bands with R_m value of Est-1 0.60, Est-2 0.40, Est-3 0.30, Est-4 (0.15). Est-1 was not inhibited by any of these three inhibitors, so this esterase zone was classified as ER (Esterase resistant to inhibitors) esterases. Est-2 and Est-3 was inhibited by Paraoxon only, but not inhibited by either Eserine or pCMB. So this zone of esterase was classified as CE (Carboxylic Esterase). Est-4 was inhibited by Paraoxon, Eserine and pCMB inhibitors, so these esterases are called as CHspesterases.

Muscle: Muscle exhibited three zones of the esterase zymogram with R_m values of Est-1 0.60, Est-2 0.40, Est-3 0.30. ESt-1 was inhibited by Paraoxon and Eserine but not with pCMB, so this zone of esterases is classified as ChE (Choline Esterase) esterases. ESt-2 and Est-3 were inhibited by all these three inhibitors, so these esterases are called CHspesterases.

Brain: Brain tissue showed three zones of esterases with R_m values of Est-1 0.60, Est-2 0.40, Est-3 0.30. Est-1 was inhibited by Eserine only but not by Paraoxon and pCMB, so this esterase is classified as Ese (Esterases inhibited by serine alone but not by op compounds) esterases. Est-2 and Est-3 was inhibited by Paraoxon and Eserine, but not with pCMB, so these esterase are called as ChE (Choline esterase) esterases.

Table 1. Tissue specific distribution of esterase in Intestine of *H. fossilis*

Intestine	Est-1	Est-2	Est-3	Est-4
Control	+	+++	+	+
Paraoxon	+	-	+	+
Eserine	+	+++	++	++
Pcmb	+	+++	++	++
Classification	ER	CE	ER	ER

CE= Carboxylesterases
ER= Esterases resistant to inhibitors

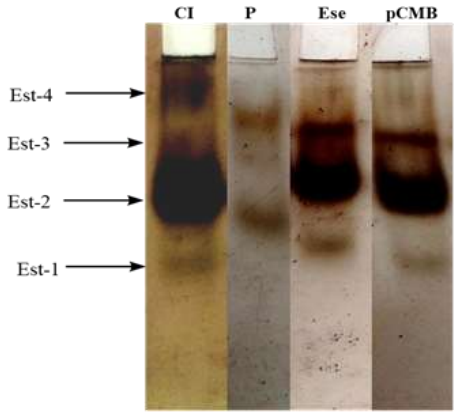

Fig. 1. Electrophoretic patterns of esterases inhibition in Intestine tissue of *H. fossilis*
CI= Control Intestine; P=Intestine tissue in the presence of Paraoxon; Ese= Intestine tissue in the presence of Eserine; pCMB= Intestine tissue in the presence of pCMB

Table 2. Tissue specific Distribution of esterase in Muscle of *H.fossilis*

Muscle	Est-1	Est-2	Est-3
Control	+	+	+
Paraoxon	-	-	-
Eserine	-	-	-
Pcmb	++	-	-
Classification	ChE	CHsp	CHsp

CHsp= Enzymes which were inhibited by Paraoxon, Esarine,and Pcmb;ChE=Cholinesterases

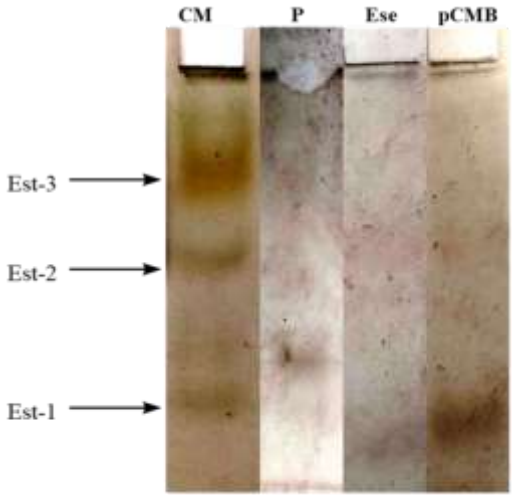

Fig. 2. Electrophoretic patterns of esterases inhibition in Muscle tissue of *H. fossilis*
CM= Control Muscle; P=Muscle tissue in the presence of Paraoxon Ese Muscle tissue in the presence of Eserine; PCMB= Muscle tissue in the presence of pCMB

Table 3. Tissue specific Distribution of esterase in Brain of *H. fossilis*

Brain	Est-1	Est-2	Est-3
Control	++	+	+
Paraoxon	+	-	-
Eserine	-	-	-
Pcmb	+++	+	+
Classification	Ese	ChE	ChE

ChE=Cholinesterases;Ese= Inhibited by Eserine alone

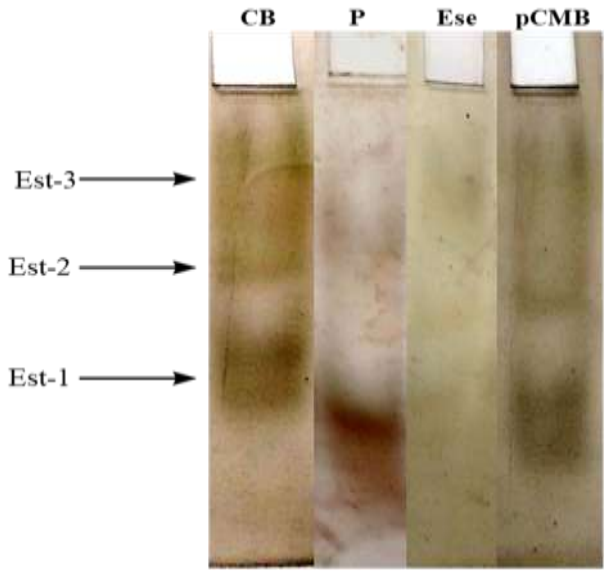

Fig. 3. Electrophoretic patterns of esterases inhibition in brain tissue of *H. fossilis*
CG= Control Brain; P=Brain tissue in the presence of Paraoxon; Ese= Brain tissue in the presence of Eserine; pCMB=Brain tissue in the presence of pCMB

4. DISCUSSION

We observed the different classes of esterases in different tissues of *H. fossilis*esterases activity is more in intestine and less in muscle of *H. fossilis.* Intestine showed ER esterases (Esterases resistant to inhibitors) and CE esterases (Carboxylesterases), muscle showed ChE (Cholinesterases) and CHspesterases (Enzymes which were inhibited by Paraoxon, Eserine, and pCMB) and brain showed ChE(Cholinesterases) and Esdp (Esterases inhibited by Eserine alone) esterases. Thomson et al.(1983 &1989) reported two classes of esterases, one a general non specificesterases catalyzing the hydrolysis of P-nitrophenyl acetate and the other hydrolyzing specifically the tyrosine esters. The substrate specificity and inhibitors sensitivity of the enzymes had been used by Holmes et al. [6], to classify the electrophoretically separated esterases found in the tissue of several vertebrates. Electrophorotic investigations of esterase, especially allozymes that acts as genetic markers in determining the taxonomic and population status of many organisms (Ferguson1980).

“Similar types of inhibition patterns revealed from the studies on esterases of fishes and othe organisms like crustaceans, insects, mollusces, and amphibians” [5], (Sujatha and Lakshmipathi, 1997; Shobha Rani & Lakshmipathi,1993; Rajaiah and Lakshmipathi, 1997; Venkaiah and Lakshmipathi.,1998; Raju& Venkaiah, 2013). “Inhibition of esterases by the organophosphates, carbamates have been used traditionally to classify them” (Alridge, 1953). “CHsp (Cholinesterase like enzymes) esterases inhibited by all these three inhibitors and Esdpesterases which are inhibited by both pCMB and Praroxon . These are implicated in biotransformation and detoxification of the pesticide” (Jokanovic, 2001; CASIDA &Quistad 2005). Different number of esterase fractions in the gut spectrum of different breeds of *B. mori* has been reported by various authors (Eguchi, Sugimoto 1965; Egorova, et al.,1977; Eremina 1985). “Esterase isozyme exhibited higher level of polymorphism in vertebrates and invertebrates” (Selander 1976). “The differences in fractions of esterase may be due to the degree of genetic heterogencity. ArEesterases were found in all the perciformes fishes but ArEesterases were not noticed in channiformes” (Rajaiahet al., 2010).

Earlier reports indicated that the esterases from vertebrates and invertebrates exhibit higher level of polymorphism. Similar types of inhibition patterns were revealed from the studies on esterases of fishes and also from other organisms like crustaceans, insects, mollusks and amphibians [22], (Bheem Rao, 2018; Swapna Ravinder Reddy, 2017, Venkaiah et al., 2013, Pranavi et al., 2012). The investigations on esterases are not clear. So far,

"Bufodienoloidsfound in the skin and glandular secretions of toads exist as multiple conjugate forms of dicarboxylic acid esters and as arginyl-dicarboxylic esters" (Schmiada and Wanbara 1979). The inhibition pattern suggests that these esterase enzymes are sensitive to organophosphate (OP) compounds, Paraoxon and Physostigmine and are classified as Carboxyl esterases [5], which are actively involved in enzyme metabolism responsible for insecticidal resistance and also in detoxification of allelo chemicals, as earlier reported. "Esterases can show post-translational modifications and formation of hybrid polymers. The band pattern also exhibits profound variation with varying electrophoretic conditions" (Gopalakrishnan, A. et al., 1997). "As a consequence of these problems, substrate specificity studies become inevitable for characterization and genetic interpretation of esterase zymograms and use of inhibitor techniques. Electrophori banding patterns of esterase isozyme in fresh water fish Channa punctatus" (Dr.M.Venkateswara Rao and Y.Venkaiah et al.,2022).Tissue esterase polymorphism of tilapia mossambica and notopterusnotopterus, V.Rajaiah,V.Vimala,et al. 2023) [28-33].

5. CONCLUSION

The esterases are involved in pesticide biotransformation and detoxification. They are useful in biotechnological applications as antidotes against poisoning and in the bioremediation of organophosphate sensors. In this present investigation CE esterases and ER Esterases are present in Intestine tissue CHsp Esterase and ChE Esterase present in muscle tissue and ChE Esterase and Ese Esterases are present in brain tissue.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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