International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 7 Issue 7 Series I ǁ July 2018 ǁ PP.18-21

Electrophoretic studies on esterase banding patterns in parotoid gland secretion and its extract of Indian toad Bufo melanostictus

Thirupathi. K and Venkaiah. Y*
Department of Zoology, Kakatiya University, Warangal- 506 009.
Corresponding Author: Thirupathi. K

ABSTRACT: *The present study has been carried out to investigate the electrophoretic studies on esterase banding patterns of parotoid gland secretion and its extract of common Indian toad (B. melanostictus). The qualitative analysis of esterase isozyme banding patterns were examined on 7.5٪ native polyacrylamide gel electrophoresis (PAGE) stained with α-naphthyl acetate as substrate. In parotoid gland secretion 3 esterase bands named as Est-1 (27.50); Est-2 (25.00); Est-3 (21.62), where as in parotoid gland extraction 6 esterase bands named as Est-1 (27.50); Est-2 (25.00); Est-3 (21.62); est-4 (35.58), Est-5 (48.50) and Est-6 (50.00) were observed with different relative mobility. The results revealed that the activity of individual esterase band was not completely inhibited by pCMB and Physostigmine (Eserine). However a complete inhibition was observed (in both parotoid gland secretion and its extract) in the presence of paraoxon (an organophosphate). Thus our present investigation reveals that all the enzymes were classified as carboxyl esterases.*

KEYWORDS:*B.melanostictus,parotoid gland secretion, α-naphthyl acetate, paraoxon, pCMB, Physostigmine, carboxyl esterase.*

DateofSubmission: 03-04-2019 Date of acceptance: 19-04-2019

I. INTRODUCTION:

Amphibians are important components of both terrestrial and aquatic ecosystems by means of their sensitivity towards environmental changes (Dullmen and Trueb, 1986; Pull et al., 2000; Marco AD et al., 2001). Amphibian skin is characterized by the presence cutaneous glands spread over the body. Basically toads have two types of alveolar glands in the epidermal layer of their skin i.e. i) mucus glans and ii) granular glands (Toledo and Jared 1995; Jared et al., 2009). Mucus glands secrete mucus substance functioning as a lubricant in the water to keep skin moist and protect the skin from the mechanical damages and prevent microbial settlement on the skin. These glands secrete glycoprotein rich material, which plays an important role in defense mechanism. The granular glands are associated with chemical defense. These gland secretions contain biogenic amines, steroids, peptides, protein, bufotoxins, oligopeptides, alkaloids in terms of pharmacological effects (Toledo et al., 1995; Eraspmer 1994; Lyttle 1996; Maciel 2003; Daly 2007; Gomes et al., 2007a). In toads these granular glands are form the parotoid glands located besides eyes and tymphanum. The venomus secretions of these parotoid glands of the toad Bufo species are known to contain several bioactive compounds (Habermehl 1995) and were used by Chinese and Japanese physicians for centuries as folk medicine (Lyttle et al., 1996; Abhishek garg 2007, Smith et al., 2005).

Preliminary survey on the venomous secretions and the extracts of gland reveal that the gland secretions contain good amount of hydrolytic enzymes, esterases. Esterases represent a diverse group of hydrolases catalyzing the formation and break down of ester bonds which are sensitive to organophosphate compounds, and play a vital role in biotransformation and detoxification of the pesticides, and are useful in bioremediation of organophosphate sensors. In present study we investigatie on the electrophoretic studies on esterase banding patterns of parotoid gland secretion and its extract of common Indian toad B. melanostictus.

Materials and Methods:

The toads (5cm to 8cm in length, weighed about 50-75grams) were collected from the vicinity of Kakatiya University hostel buildings, Warangal, Telangana State. The parotoid glands were gently pressed to release the secretions(Linde & Myer-1971). The secretions were collected in ice-jacketed containers. After collecting the secretions, the gland was dissected out and blotted free of blood clots and other adherent tissues and weighed to the nearest milligram. The gland as well as the secretions were homogenized in (10٪) 0.01M Tris-HCL Buffer (pH 7.4) containing 0.9٪ NaCl.

The homogenates were centrifuged and the supernatants were diluted 1:1 with 20% sucrose containing 0.01٪ bromophenol blue as tracking dye. An aliquot of 0.1ml of these solutions were loaded directly on to the separating gel. Esterase patterns were separated on thin layer (1.5mm thickness) polyacrylamide gels (7.5٪,

10X10 cm). The gel mixture was prepared according to the procedure of Clarke. Gelling was allowed for 45 minutes. After loading on to the gel, the samples were over laid with electrode buffer and 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 minutes followed by 150 volts for the rest of the run was supplied during electrophoresis. The electrophoretic run was terminated when the tracking dye migrated to the distance of 8 cm from the origin. Esterases were visualized on the gels by adopting the staining procedures of Holmes and Masters, (1967) and described by Reddy and Laksmipathi, (1988). Physostigmine (10^{-4}M), pCMB (Parachloro Mercuric Benzoate) (10^{-3}M) and Paraoxon (0, 0-di-ethyl-4-nitrophenyl phosphate) ($2x10^{-3}$M) were used for inhibitor sensitivity studies. The gels were pre-incubated in the buffer containing the above concentrations of inhibitors for half an hour. Then they were stained for esterase activity with 1- napthylacetate as substrate. The inhibitors in concentrations used for pre incubation were added to the stain buffer to prevent reversal of inhibitory action of the compounds. The relative mobility (R_m) activity of zone was determined according to Klebe (1975). The esterase activity of the different bands obtained from parotoid gland secretion and its extract was arbitrarily measured based on eye estimation of staining intensity, categorized into deep stained (DS), medium deep stained (MDS) and faint stained (FS).

Characterization of enzymes in parotoid gland secretion and its extract of B. melanostictus

II. RESULTS :

The results obtained on the electrophoretic studies on esterase banding patterns in parotoid gland secretion and its extract of toad Bufo melanostictus are presented, the details about Relative mobilities (R_m values) of individual esterase zone, visual end points and classification of these esterases are presented in Table.1, fig.1. and table.2, fig. 2. α-naphthyl acetate was used as a substrate to score the intensity of esterase bands.

The results indicated that the parotoid gland secretion (ta.1 & fig.1) contained 3 hyperactive slow moving cathodal bands named as Est-1, Est- 2, Est- 3 with R_m values 27.50 , 25.0 and 21.6 respectively. Est-2 was deep stained in pCMB (+++), Est-3 was medium stained (++) and Est-1 was faint stained (+). The parotoid gland extract contain six bands (tab.2 & fig.2) named as Est-1, Est- 2, Est- 3, Est-4, Est- 5 and Est- 6 with R_m values 27.50, 25.0, 21.62, 35.58, 48.50 and 50.00 respectively. Est-1, Est- 2, Est- 3 were deeply stained in pCMB (+++); Est- 5 was medium stained in pCMB and Eserine; Est-6 was faint stained in pCMB and Eserine, both were slow moving bands. Three were fast moving anodal bands and two were slow moving cathodal bands and remaining one band were found in middle zone band in the parotoid gland extract.

From the observation of esterase inhibition study, reveals that the parotoid gland secretion of toad esterase bands had heavy deposition and completely inhibited by pCMB and Physostigmine (Eserine), but not effected by Paraoxon so they are classified as Carboxyl esterases. In parotoid gland extract the heavy deposition of esterase bands classified as Carboxyl esterases because the total inhibition was not observed in presence of pCMB and Eserine. Paraoxon inhibited the activity of all esterases found in the gland secretion and its extract of B. melanostictus.

The inhibition patterns suggest that the enzymes are sensitive to organophosphate, paraoxon. So all the enzymes are classified as Carboxyl esterases

Tab.1 Classification of individual esterase zones in parotoid gland secretion of B. melanostictus

	Est-1 (27.50)	Est-2 (25.00)	Est-3 (21.62)
PGS	+++	+++	+++
pCMB	+	+++	++
Eserine	+	+++	++
Paraoxon	-	-	-
Classification	**C.E**	**C.E**	**C.E**

PGS=Parotoid Gland Secretion, CE= carboxyl Esterase
pCMB= Para Chloro Mercuric Benzoate

Tab.2 Classification of individual esterase zones in parotoid gland extract of B. melanostictus

	Est-1 (27.50)	Est-2 (25.00)	Est-3 (21.62)	Est-4 (35.58)	Est-5 (48.50)	Est-6 (50.00)
PGE	++	+++	+++	+	++	+
pCMB	+++	+++	+++	+	++	+
Eserine	+	++	++	+	++	+
Paraoxon	-	-	-	-	-	-
Classification	**C.E**	**C.E**	**C.E**	**C.E**	**C.E**	**C.E**

PGE=Parotoid Gland Extract Est= Esterase
CE= Carboxyl Esterase pCMB= Para Chloro Mercuric Benzoate

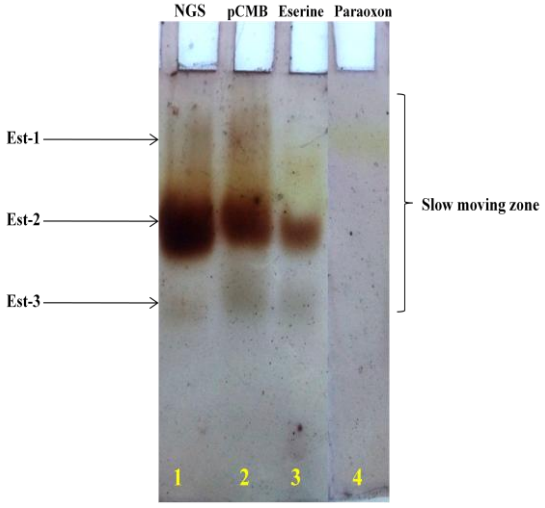

Fig.1. Parotoid gland secretion in presence of three inhibitors (pCMB, Eserine, Paraoxon)

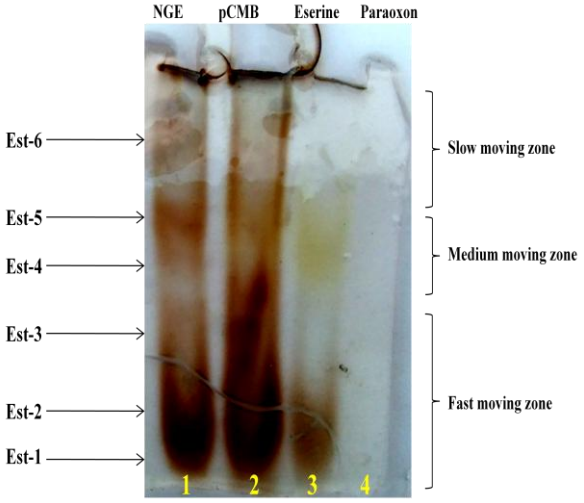

Fig.2. Parotoid gland extract in presence of three inhibitors (pCMB, Eserine, Paraoxon)

III. DISCUSSION:

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 (Bheem Rao, 2018; Swapna Ravinder Reddy, 2015, 2017, Venkaiah et al., 2013, Pranavi et al., 2012).

The investigations on esterases are not clear. So far, Bufodienoloids found 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 (Reddy and Pathi, 1988), 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.

IV. CONCLUSION:

The esterases are implicated in biotransformation and detoxification of the pesticides they have importance in biotechnological applications as antidotes against poisoning and are useful in bioremediation of organophosphate sensors

ACKNOWLEDGEMENT:

The author expresses their deep gratitude to the Head of the Department of Zoology, Kakatiya University for providing logistic Support.

Conflict of interest:

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

REFERENCES:

[1]. Duellman, W. E., and L. Trueb. 1986. Biology of Amphibians. Mcgraw-Hill Book Co.: Hightstown, N.J., USA; London, England.
[2]. Toledo R.C and Jared.C Cutaneous granular glands and amphibian venoms. Comparative Biochemistry and Physiology, 111(1),1995,1-25.
[3]. Jared C, Antoniazzi M M, Jordao AEC, Silva J RMC, Greven H, and Rodrigues MT. Parotoid macroglands in toad (Rhinella jimi): their structure and functioning in passive defence. Toxicon., 2009; 54(3): 197-207.
[4]. Erspmer, V. and Melchiorries.Active polypeptides of the amphibian skin and their synthetic Analogues.Pure and Appl.Chem, 35, 1973, 463-494.
[5]. Lyttle, T., Goldstein, D. and Gartz, J.: J. Psychoactive Drugs., 28(3): 267-290 (1996).
[6]. Maciel NM, Schwartz CA, Rodrigues Pires JO, Sebben A, Castro MS, Sousa MV, Fontes W, Ferroni Schwartz EN.2003. Composition of indolealkylamines of Bufo rubescenscutaneous secretions compared to six other Brazilian bufonids with phylogenetic implications. Comparative Biochemistry and Physiology B,134(4):641- 649.

[7]. Daly JD, Wilham JM, Spande TF, Garraffo HM, Gil RR, Silva GL, Vaira M 2007. Alkaloids in bufonid toads (Melanophryniscus): temporal and geographic determinants for two Argentinian species, Journal of Chemical Ecology, 33(4): 871-887.
[8]. Gomes A, Giri B, Saha A, Mishra R, Dasgupta SC, Debnath A. 2007a. A Bioactive molecules from amphibian skin: their biological activities with reference to therapeutic potentials for possible drug development. Indian J. Exp. Biol., 45:579-593.
[9]. Habermehl.GG. Antimicrobial activity of amphibian venoms. Studies in natural products chemistry, part-C, 1995, 327-339.
[10]. Garg, A.D., Kanitkar, D. V., Hipargi, R.V and Gandhare. A.N.(2007). Antimicrobial activity of skin secretions isolated from Indian toad, Bufo melanostictus Schneider 1799. Nature Precedings, DOI: 10.1038/npre.2007.1204.1
[11]. Rollins-Smith, L.A., Reinert, L.K., O'Leary, C. J., Houston. L. E and Woodhams, D. (2005). Antimicrobial peptide defenses in amphibian skin. Integr. Comp. Biol., 45: 137-142. PMID: 21676754.
[12]. Meyer K, Linde H. Collection of toad venoms and chemistry of toad venom steroids.In: Bucherl W, Buckley E, editors, Venomous animals and their venoms.NY. Academic Press, 2, 1971, 521–556.
[13]. Clarke. Simplified "Disc" (Polyacrylamide Gel) Electrophoresis. Ann N Y Acad Sci. Dec 28; 121: 1964, 428–436.
[14]. Holmes RS, Masters CJ.1967. The developmental multiplicity and isoenzyme status of cavian esterases. Biochim Biophys Acta., 132(2):379-399.
[15]. Reddy, T.M. and Lakshmipathy,V.: Curr. Sci., 57(1): 24-27. (1988).
[16]. Klebe J. 1975 A simple method for the quantitation of isozyme patterns. Biochem. Genet. 13, 805-812.
[17]. T. Bheem Rao, K. Thirupathi and Y. Venkaiah Comparative Study Of Electrophoretic Patterns Of Esterases In Various Tissues Of Fresh Water Cat Fish Heteropneustes Fossilis (Bloch).. Br J Pharm Med Res , Vol.03, Issue 01, Pg.840-845, January - February 2018. ISSN:2456-9836 Cross Ref DOI : https://doi.org/10.24942/bjpmr.2018.201
[18]. Swapna P. and Ravinder Reddy, T. 2015. Esterase Variability in Different Tissues of Flying Frog (Rhaco Phorus Lateralis) through Polyacrylamide Gelelectro Phoresis, International Journal of Pharma Research & Review, 4(4):7-12.
[19]. Reddy et al. Electrophoretic Patterns of Esterases from Different Tissues of Arion Hortensis. Int J Pharma Res Health Sci. 2017; 5 (1): 1563-1566.
[20]. Raju and Venkaiah. Electrophoretic Patterns of Esterases of Parotoid Gland of common IndianToad Bufo melanostictus (Schneider), Journal of Cell and Tissue Research, 2013 Vol. 13(1) 3491- 349.
[21]. Pranavi S, Prasad MSK and Lakshmipathi V. 2012. Patterns of Esterases in the Developing Stages of the Red Flour Beetle, Tribolium castaneum. International Journal of Plant, Animal and Environmental Sciences, 2(4):60-64.
[22]. A. GOPALAKRISHNANi, KULDEEP K. LAL AND A.G. PONNI.Esterases in Indian major carps - 'Rohu' (Labeo rohita) and 'Mrigal' (Cirrhinus mrigala) (Teleostei, Cyprinidae). Indian J. Fish.. 44(4) : 361-368, Oct.-Dec, 1997.

Thirupathi. K" Electrophoretic studies on esterase banding patterns in parotoid gland secretion and its extract of Indian toad Bufo melanostictus" International Journal of Pharmaceutical Science Invention(IJPSI), vol. 07, no. 07, 2018, pp. 18-21