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RESEARCH ARTICLE

EFFECT OF PHYSOSTIGMINE (CARBAMATE) ON ELECTROPHORETIC PATTERNS OF ESTERASE ACTIVITY OF PAROTOID GLAND SECRETION AND ITS EXTRACT OF COMMON INDIAN TOAD *BUFO MELANOSTICTUS* (SCHNEIDER)

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ABSTRACT

The present study was carried out to investigate the effect of physostigmine (a carbamate pesticide) on the electrophoretic patterns of esterase activity in parotoid gland secretion and its extract of common Indian toad *Bufo melanostictus* (Schneider). The patterns of the esterases were examined on 7.5% of polyacrylamide gel electrophoresis (PAGE) stained with α- napthyl acetate. The parotoid gland secretion and its extract were exposed to the toxicant physostigmine and the variations were observed on the activity of esterase banding patterns at different time intervals i.e., 4 hours, 8hours, and 12 hours. The findings that the parotoid gland extract showed homology in esterase bands with more intensity compared to than the parotoid gland secretion.

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INTRODUCTION

Amphibians are sensitive species in agro ecosystems. Their highly permeable skin and sensitivity to agrochemicals make these vertebrate group excellent models for studying the impacts of pesticides on an ecosystem. It is necessary therefore, to develop biochemical biomarkers of pesticide exposure that predict these adverse effects at individual and population levels. Most biomarkers used in field monitoring provide an indication of pollutant exposure only. The use of a suite of biomarkers becomes a recommended strategy for assessing the impact of pesticides on amphibians (Lajmanovich *et al.,* 2008). The skin of the amphibians is characterized by the presence of cutaneous glands present over entire body of the skin (Juliana Mozer Sciani *et al.,* 2013). The skin plays a vital role for survival of amphibians such as respiration, water regulation, anti- predator and antifungal defense (Jared *et al.,* 2009). The amphibian skin consists of two types of glands mucus and granular glands both of them are alveolar or acinar glands (Pedro *et al.,* 2013; Daly *et al.,* 2007; Maicel *et al.,* 2003). The mucus glands secrete a mucin substance which is responsible for keeping the skin moist and slippery and protect the skin from the mechanical damages and prevent microbial settlement on the skin. The granular glands are responsible for production of the noxious (or) toxic secretion. Those glands are scattered over the surface of the skin (Jared *et al.,* 2009; Gomes *et al.,* 2007a; Clarke, 1997). These granular gland secretions contain rich components like bufogenins and bufotoxins (steroids) biogenic amines, alkaloids and peptides. These chemical defense can be directed either against the predators and microorganisms (Siano *et al.,* 2014). The toad venom called Chansu, the traditional Chinese medicine (TCM) obtained from the skin and parotoid venom glands of the toad (*Bufo bufo gragaizans*) cantor and *B. melanostictus* (Schneider). This venom is frequently used as an effective clinical TCM preparation to treat the malignant tumors (Jing Zhou *et al.,* 2015, Gomes *et al.,* 2010).

The Physostigmine is a natural carbamate derivative pesticide. The exposure of carbamate that acts through acetylcholine esterase inhibition may serve as model chemical for neurotoxins (Mirjana B Colovic *et al.,* 2013). The effect of pesticide stress showed a constant decrease in the levels of metabolic enzymes. Carbamates are considered as reversible AchE inhibitors and analogous to OPs and reversibly inhibit neuropathy target esterases.

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The present study was aimed to investigate the effect of Physostigmine (a carbamate pesticide) on electrophoretic patterns of esterase activity of parotoid gland secretion and its extract of common Indian toad, *Bufo melanostictus*.

MATERIALS AND METHODS

The toads (7cm to 10cm in length, weight about 50-75grams) were collected from the vicinity of Kakatiya University hostel buildings, Warangal, Telangana State, India. The parotoid glands were gently pressed to release the secretion. The secretion was collected in ice- jacketed containers. After collecting secretions, the gland was dissected out, blotted to free from blood clots and other adherent tissue and weighed. The tissue was crushed in 0.01M Tris-Hcl Buffer (pH 7.4). This homogenate as well as the secretion of gland 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 aliquote of 0.1ml of these solutions was loaded directly on to the separating gel. Esterase patterns were separated on thin layer (1.5mm thickness) polyacrylamide gels (7.5%). The gel mixture was prepared according to Clarke (1964). Gelling was allowed for 45minutes. After loading on the gel, the samples were overload 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 15minutes 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 8.0 cm from the origin. Esterases were visualized on the gels by adopting the staining procedure of Holmes and Masters (1967); Reddy and Lakshmipathi (1988).

Preparation of physostigmine (carbamate) concentration for induction

To observe the electrophoretic patterns of esterase variations in *B. melanostictus.* The pesticide Carbamate (Physostigmine) (10^{-4} M) and normal saline, were injected intradermally into parotoid gland contra laterally (Raju and Venkaiah, 2014). The *in vivo* effects of Physostigmine in esterase band variations of parotoid gland secretion and its extract of *B. melanostictus* were studied on the gels by adopting the staining procedure of Holmes and Masters (1967) at different time intervals i.e., 4,8 and 12hours.

RESULTS AND DISCUSSION

The results obtained on the effect of Physostigmine (Carbamate) on the electrophoretic patterns of esterase of parotoid gland secretion and its extract of common Indian toad *Bufo melanostictus* are presented in Fig.1 and 2 respectively. The details about the relative mobility of individual esterase zones are presented in Table. 1. α-napthyl acetate was used as substrate to score the activity of esterase on gels. Results showed differences at different concentrations of the parotoid gland secretion and its extract which were followed by visibility of the zones in electrophoresis. The relative mobility of individual esterase band indicates (Table.1 & Fig.1) that the Rm values were 28.57, 35.85 and 42.85 in the slow moving zone (region). The esterase patterns obtained in Fig.1 indicated that the parotoid gland secretion showed a single hyper active band in 8 hours and in 12 hours, a band of moderate intensity and in 4 hours a band with low intensity.

The patterns of parotoid gland extract observed indicates that the Rm values of the esterase bands were 26.66, 35.85, 53.33, 57.14 and 66.66. A band with Rm value 26.66 had high intensity at all time intervals except at control (Fig.2). The parotoid gland extract (Fig. 2) contained double esterase band with Rm value 53.33, 57.14, 66.66 (Table 1) in the middle region. The pattern observed indicated that the parotoid gland secretion has low intensity compared to parotoid gland extract but with minor homogeneity in esterase banding pattern.

Effect of Physostigmine on Electrophoretic patterns of Esterase activity

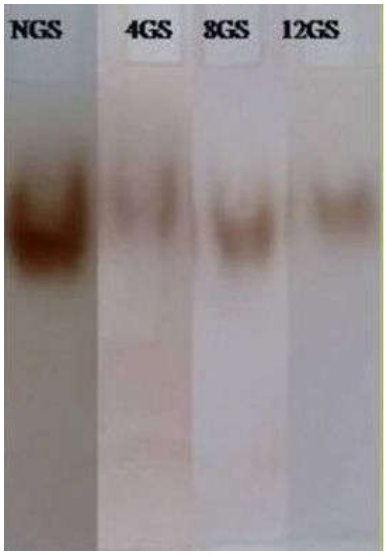

Fig.1. Electrophoretic bands indicating NGS= Normal gland secretion, 4GS=4H gland secretion, 8GS=8H gland secretion and 12GS=12H gland secretion

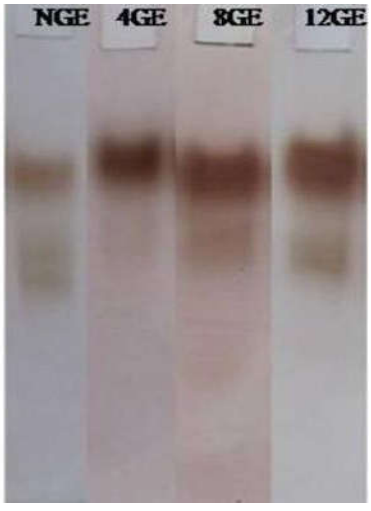

Fig.2. Electrophoretic bands indicating NGE= Normal gland secretion, 4GE=4H gland extract, 8GE=8H gland extract and 12GE=12H gland extract

Table 1. Effect of Physostigmine on Electrophoretic patterns of Esterase activity of Parotoid gland secretions and its extract of *Bufo melanostictus*

	Rm Values							
	26.66	28.57	35.85	40.00	42.85	53.33	57.14	66.66
Gland Secretion		+++	+++		+++			
4H Gland Secretion		++	++		++			
8H Gland Secretion		+++			+++			
12H Gland Secretion		++	++		++			
Gland Extract	+++		+++			+++	+++	+++
4H Gland Extract	+++		++			+	++	+
8H Gland Extract	+++		+++			+++	+++	+++
12H Gland Extract	+++		+++			+++	+++	+++

Rm (Relative mobility) shown as percent migration of the zone from the origin to that of tracking dye; +++= indicates the high activity; ++=indicates the moderate activity; +=indicates low activity; (-) =indicates no activity.

The present results clearly indicated that the parotoid gland secretion and its extract showed high intensity in normal compared to different time intervals. Esterases were shown to have a role in xenobiotic metabolism of drugs and foreign chemicals (Satoh, 2005). They are used as catalyst in the synthesis of specific optical isomers in fine chemical industries (Patel, 2000; Faber, 2000). In view of the above results the esterases can be used as tools in establishing the genetic relatedness among the closely related species (Wu and Wu, 1983). Enzymes such as esterases (E.C.3.1.X) represent a diverse group of hydrolases catalyzing the formation and breakdown of ester bonds. Inhibition of esterases by the organophosphates, carbamates had been used traditionally to classify as A and B-esterases (Aldridge, 1953). Various authors reported on the patterns of esterases in common Indian Toad *Bufo melanostictus*, and flying frog *Racophorus* (Raju and Venkaiah, 2013; Swapna and Ravinder Reddy 2015; Balen *et al.,* 2003a]. The plama β-esterase activity has been used in several studies to monitor vertebrate wildlife exposed to pesticides (Chuiko *et al.,* 2003; Sanchez-Hernandez, 2006, Wheelock *et al.,* 2008). Particularly Butryl cholinesterase (BchE), Carboxyl esterase (CbE) were useful indicators of amphibian exposure to anti chE chemicals (Lajmanovich *et al.,* 2004, 2008; Attademo *et al.,* 2007). Carbamates and OP pesticides usually inhibit these enzymes (Dettbarn *et al.,* 1999; Sogorb and Vilanova 2002; Wheelock *et al.,* 2004, 2008). Khan *et al.,* (2003, 2005) determined that the pyrethroid pesticides inhibit the BchE activity of Anuran (*Rana cyanophlystis*) reptile (*Calotes versicolor*) species. Mor and Ozmen (2010) demonstrated cholinesterase activity inhibition induced by esterase enzyme in rabbit.

The differences in fractions of esterase may be due to the degree of genetic heterogeneity. The heterogeneity of esterases from several species of insects has been demonstrated employing electrophoretic techniques and they are shown to be tissue specific (Raju *et al.,* 2013). Recent studies on esterases from several species implicate them to be involved in the regulation of juvenile hormone titre in the haemolymph (Pranavi *et al.,* 2012). The *in vivo* functions of esterases are not clear. *Bufodienoloides* found in the skin and glandular secretion of toad exists as multiple conjugate forms of dicarboxylic acid esters and as arginyl carboxylic esters. Thus we conclude that the esterase isozyme could essentially be used as a marker to understand genetic makeup of a species for any conservation effort as well as species identification and selection.

Conclusion

The present study indicate that the variability of patterns in esterase isozymes to describe the electromorphs of an individual representative. It is possible that the enzymes may play a critical role in processing and potentiating toxins secreted by venomous glands and whether the esterases present in the secretions have a role in de-esterifying these esters which needs further investigation.

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