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Original Article

Comparative Study of Electrophoretic Patterns of Proteins in the Parotoid Gland Secretion and its Extract of *Bufo melanostictus* (Schneider) through SDS-PAGE and Urea SDS-PAGE

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Abstract

The present study was under taken to analyze the qualitative analysis of the comparative study of eletrophoretic patterns of proteins in the parotoid gland secretion and its extract in terrestrial toad *Bufo melanostictus* (Schneider). The protein patterns indicated that the secretion has less number of protein bands compared to the gland extract. The patterns of protein bands were observed in the parotoid gland extraction of *B. melanostictus* through Sodium Dodecyl Sulphate and Poly Acrylamide Gel Eletrophoresis (SDS-PAGE) indicated a distinct of four protein bands and some additional bands with poor resolution and was compared with Urea SDS-PAGE. The protein bands indicated a distinct of six protein bands with some other additional bands in Urea SDS gels. In the parotoid gland secretion two protein bands in SDS-PAGE, where as four protein bands were observed in the parotoid gland secretion of *B. melanostictus* through Urea-SDS PAGE. The protein subunit patterns were identified by using standard marker protein and R_m values were calculated accordingly. The eletrophoretogram both the SDS-PAGE & Urea SDS-PAGE patterns of parotoid gland secretion and its extract showed homology in protein bands with minor variations.

Keywords: *Bufo Melanostictus*; Parotoid Gland; Protein Patterns; Urea SDS PAGE; Electrophoresis.

Introduction

Amphibians are treated as bio-indicators of aquatic and terrestrial ecosystem owing to their sensitivity to changes in the environment [1, 2, and 3]. Amphibians like toads are characterized by the presence of cutaneous glands spread over the skin. Basically two different types of glands developed in the amphibian skin i.e., I) Mucus secreting glands generally associated to maintain the humidity and cutaneous respiration and to protect the skin from mechanical damages and prevent microbial settlement on the skin; these glands secrete glycoprotein rich material which plays an important role in defense mechanism. II) Granular glands generally associated with chemical defense against predators and microbial infection [4-6]. The product secreted by such glands contain a wide variety of rich components like biogenic amines, bufo toxins, oligo peptides, proteins, guanidine derivatives, steroids and alkaloids in terms of pharmacological effects [4, 7-9]. The epidermal glands in amphibians are more evolved and are alveolar glandular cells and open on to the surface of the skin through ducts. In toads these glandular cells form the parotoid glands located between eyes and tympanum [8, 10]. The venomous secretions of the parotoid glands of the *Bufo* species are known to contain several bioactive compounds [11] and were used by Chinese, Indian traditional medicinal practice and Japanese physicians for centuries as folk medicines like “kyushin” and “Chan Su” [8,11]. The granular secretions are known to be secreting a variety of compounds which are species specific [12, 13].

The toad *Bufo melanostictus* (Schneider) is a very common amphibian in India So far, there are few reports on the protein patterns of *B. melanostictus* through Urea-SDS-PAGE. The present investigation has been undertaken for the comparative study of electrophoretic patterns of proteins in the parotoid gland secretion and it’s extract of *Bufo melanostictus* (Schneider) through Urea and SDS-PAGE in order to understand their possible defense role against

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microbial infections.

Material and Methods

Animal Materials Chosen for Study

The toads (7cm to 10cm in length, weighing about 45-70 grams) were collected from the vicinity of Kakatiya university hostel buildings, Warangal, Telangana, India.

Extraction and Collection of Samples

The parotoid glands were gently pressed to release the secretions. The secretions were collected in ice-jacketed containers. After collecting the secretions the gland was dissected out and were blotted free of blood clots and other adherent, tissues were weighed to the nearest milligram and gland as well as secretions were homogenized (10%) in 0.01M Tris-HCl buffer (pH 7.0) containing 0.1% Sodium Dodecyl Sulphate (SDS) and 0.9% NaCl the extracts were centrifuged at room temperature (30±2°C).

Experimental Procedure

The electroporetic patterns of proteins of parotoid gland secretion and its extract of *Bufo melanostictus*(scheinder) using SDS-PAGE and Urea-SDS-PAGE were performed through methods as described by Lammeli's [14] and Anderson et al.[15]. Thin layers (1.5mm thick) polyacrylamide slab gels were prepared by using the glass plates. The protein for electrophoretic studies was extracted by homogenizing the parotoid gland secretion and its extract (10%) in 0.01M Tris-HCl buffer pH (7.0) containing 0.1% sodium dodecyl sulphate (SDS) and 0.9% NaCl. The extracts were centrifuged at 2,000 rpm for 20 minutes in a clinical centrifuge at room temperature (30±2°C) and the supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS, mercaptoethanol and bromophenol blue as the tracking dye; 0.1 ml (5 mg) of the parotoid gland secretion and its extract was loaded on to the separating gel directly. The electrode buffer, 0.025 M Tris and 0.192 M glycine, was used for Laemmli's method, whereas 0.074 M Tris, 0.1% SDS adjusted to pH 7.8 with concentrated HCl as upper chamber buffer and 1M Tris, 0.2 % SDS adjusted to pH 7.8 with concentrated H_2SO_4 for the Urea SDS-PAGE. A constant current of 50 volts for the first 15 minutes followed by 150 volts for the rest of the run was applied to the gel. The current supply was terminated when the tracking dye migrated to a distance of 8 cm from the origin. A solvent containing 0.25% Coomassive brilliant blue in methanol: water: acetic acid (5:5:1) was used for the staining proteins separated on gel by Laemmli's method. Silver nitrate [16] was used for staining proteins separated by the method of Andeson et al [15].

Standardization of Protein Bands

The molecular weight standards were used in comparing the variations noticed in the SDS-PAGE were the low molecular weight protein standards (14 to 66 KDa) from the SIGMA-Chemical company from USA and the Urea-SDS-PAGE were of molecular weight protein standards (14 to 200 KDa) from the Bio-Rad-Chemicals company from USA.

Results

The protein patterns of *Bufo melanostictus* observed in parotoid gland extract and its secretions and their relative mobility (Rm) are presented in Fig.1 and Table 1 respectively. The protein patterns observed on SDS-PAGE stained with Coomassie brilliant blue indicated distinct differences in the mobility of some bands of the parotoid gland extract and its secretion. Comparison of the protein bands of various regions with standard marker proteins revealed that the variation is higher in the regions of slow moving zones "A" (mol wt.66KDa) and those with fast moving zones "C" (mol.wt. 24KDa,14.2KDa). The pattern obtained in the middle region "B" (mol.wt. 45KDa, 36KDa) is more (or) less similar in secretion and gland extract.

The electrophoretogram obtained revealed that there is a decrease in the intensity of protein bands of parotoid gland secretion compared to protein bands of parotoid gland extraction. A protein band with Rm value 0.11 (nearer to molecular weight 66 KDa) showed decrease in the intensity in parotoid gland secretion whereas high intensity in parotoid gland extraction. The Rm values of protein bands 0.12, 0.21, 0.25 in between the molecular weight 66 KDa-45 KDa completely disappeared in parotoid gland secretion (Zone. A) in slow moving zone compared to parotoid gland extraction. The Rm value of protein bands 0.31, 0.50 and 0.52 in between the molecular weight of 45 KDa-35 KDa were completely absent in parotoid gland secretion (Zone. B) in the middle region compared to parotoid gland extraction. The protein bands of Rm value of 0.84 was absent in the parotoid gland secretion, whereas a protein band with Rm value 0.85 was absent in the parotoid gland extraction nearer to molecular weight 14.5 KDa in the fast

moving zone (Zone. C).

The patterns of proteins of parotoid gland extract and its secretion on Urea SDS-PAGE indicated a less number of protein bands in parotoid gland secretion with decrease in the intensity compared to parotoid gland extraction. In the slow moving zone "A" (mol.wt.200-97KDa) a protein band with Rm value 0.21 (molecular weight 116KDa) showed low intensity in parotoid gland extract and not observed in parotoid gland secretion. A protein band with Rm value 0.28 (molecular weight 97 KDa) was observed in both parotoid gland extract and its secretion. The Rm value 0.42 protein band was observed only in parotoid gland extract and the Rm value 0.45 protein band was observed only in parotoid gland secretion in between the molecular weight 66-45KDa.Protein bands with Rm values 0.54, 0.60 observed in parotoid gland extract and the Rm with 0.58 in parotoid gland secretion nearer to the molecular weight 45KDa in the middle region "B" (mol.wt. 66-45KDa). In the fast moving zone "C" (mol.wt. 31-14KDa) the Rm value with 0.68 (molecular weight 31KDa) was observed in the parotoid gland extract which was disappeared in parotoid gland secretion. The Rm value 0.78 (molecular weight 22KDa) was observed in parotoid gland secretion and disappeared in gland extraction. The Rm value 0.85 (molecular weight 14KDa) was observed in both parotoid gland secretion and its extract (Zone. C).

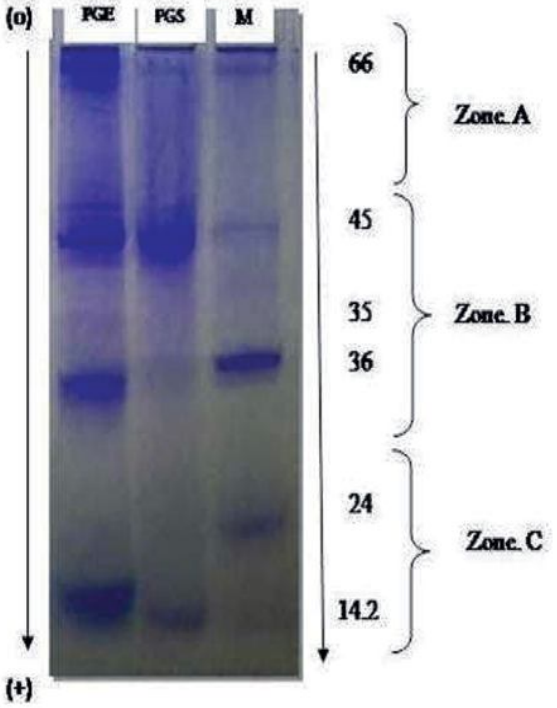

Figure-1 SDS-PAGE Electrophoretic Patterns of Proteins of *Bufo melanostictus*
Parotoid Gland Extract and Secretion stained with Coommassie brilliant blue.
Left lane indicates (M) mol. weight strands (66-14.2 KD.) 'A', 'B', 'C' zones.
PGE = Parotoid gland Extract.
PGS = Parotoid gland Secretion.
M = Molecular weight standards (14 to 66 KD).
Zone A = mol. wt. 66 KD.
Zone B = mol.wt.45 KD, 36 and 35 KD.
Zone C = mol.wt. 24 KD, 14.2 KD.
O = origin.
(+) = Anode.
↓ = Direction of run.

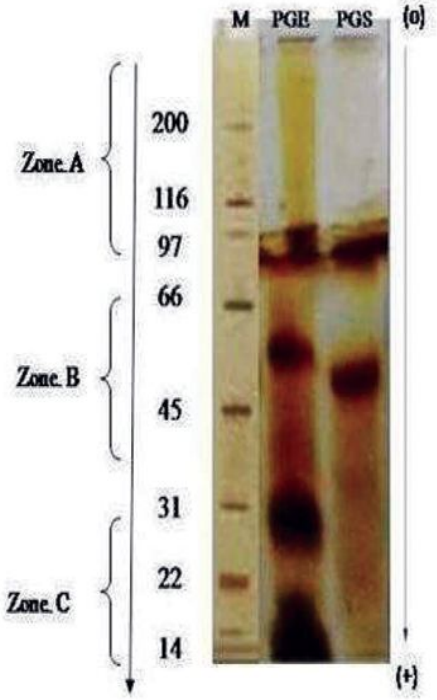

Figure -2 Urea-SDS-PAGE Electrophoretic Patterns of Proteins of *Bufo melanostictus*
Parotoid Gland Extract and its Secretion stained with Silver nitrate
Right lane indicates (M) mol. weight strands (200-14 KD.) 'A', 'B', 'C' zones,
PGE = Parotoid gland Extract,
PGS = Parotoid gland Secretion,
M = Molecular weight standards (14 to 200 KD),
Zone A = mol. wt. 200 KD, 116 KD and 97 KD
, Zone B = mol.wt.66 KD and 45 KD,
Zone C = mol.wt. 31KD, 22 KD and 14 KD;
O = Origin,
(+) = Anode,
↓ = Direction of run.

Table 1: Rm values of parotoid gland secretion and extract of Bufo melanostictus (Scheinder) on SDS-PAGE

Marker	Parotoid Gland Extract	Parotoid Gland Secretion
.07	0.07	0.07
-	0.11	-
-	0.12	-
-	0.21	-
-	0.25	-
0.28	0.28	0.28
-	0.31	-
0.50	0.50	-
0.52	0.52	-
0.64	-	-
-	0.84	-
0.85	-	0.85

Table 2: Rm values of parotoid gland secretion and extract of Bufo melanostictus (Scheinder) through Urea SDS-PAGE

Molecular Marker Standards	Parotoid Gland Extract	Parotoid Gland Secretion
0.14	-	-
0.21	0.21	-
0.28	0.28	0.28
0.35	-	-
-	0.42	-
-	-	0.45
0.50	-	-
-	0.54	-
-	-	0.58
-	0.60	-
0.68	0.68	-
0.78	-	0.78
0.85	0.85	0.85

Discussion

The patterns of protein bands observed in the parotoid gland extract and its secretion of the toad on SDS-gel indicated a distinct of four protein bands with several additional bands with poor resolution, exhibiting minor variations in the slow moving zone whereas a distinct of two protein bands were observed in the parotoid gland secretion. Therefore, the protein patterns observed in the parotoid gland extract and its secretion are more or less similar with minor variations.

The presence of protein bands with identical mobility in the secretions and gland extracts, indicate the similarity of proteins secreted probably by granular cells of epidermis. When the gland is pressed the secretion is released in the form of sticky fibrillar material [17]. Various authors have reported that the alkaloids and steroids present in *Bufo* as toxic and anti feeding agents, acting as a major chemical defense strategy against predators, and also act on the cardiovascular system by raising the blood pressure and / or increasing the contraction force of the heart [18-22]. The secretary proteins exist as coiled filaments within epidermal granular cells [23]. The presence of these arrays of proteins in Bufo parotoid gland secretions suggests a more complex role for these secretions than simply anti-predator defense. The peptides found in various species of toads and frogs which possess antimicrobial activities are of a much smaller molecular size range than encompassed by SDS-PAGE as used here. For instance, the magainins found in skin secretions of *Xenopus* are typically of 21-26 amino acid residues in length [24].

The electrophoretogram obtained from parotoid gland extract and its secretions of the protein patterns through Urea SDS-PAGE in *Bufo melanostictus* and their relative mobilities are presented in Figure 2 and Table 2, respectively. The protein patterns observed on Urea SDS-PAGE stained with Silver nitrate stain indicated distinct differences in the mobility of some bands of the parotoid gland extract and its secretion. The protein band comparison of various regions with standard marker proteins revealed that the variation is higher in the fast moving zone "C" (mol.wt. 31-14KDa) and those with middle region "B" (mol.wt. 66-45KDa). The pattern observed in the slow moving zone "A" (mol.wt. 200-97KDa) is more or less similar in the secretion and gland extract.

The pattern of proteins observed in the parotoid gland extract and its secretion of toad observed on Urea-SDS gel indicated a distinct of six protein bands and some additional bands with weak staining on the gel in fast moving zone, whereas a distinct of four protein bands were observed in the parotoid gland secretion and one additional band with weak staining on the gel in slow moving zone. Therefore, the protein patterns of parotoid gland extract as well as the gland secretions of the toad exhibited some regions of similarity (Fig 2).

Conclusion

The results of present investigation reveals that the analysis of protein patterns of *Bufo melanostictus* on SDS-PAGE and Urea-SDS gel, in spite of some minor differences in the total protein concentration and

relative concentration within the same sample, would lead to the conclusion that the secretions are very similar among themselves in *Bufo melanostictus.*

In view of the above results it can be concluded that the exudates of toad parotoid gland revealed that the presence of protein bands with identical mobility both in secretion and gland extract indicating homology of cell lines and its secretion in *B. melanostictus*.

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