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Research Article

PROTEIN PATTERNS OF TISSUE AND MUCUS EXTRACT OF ARION ATER (BLACK SLUG) THROUGH UREA-SDS-PAGE

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ABSTRACT:

Objective: The present investigation has been under taken to study the electrophoretic banding patterns of proteins in the tissue and mucus extract of Arion ater (Black slug).

Methods: Urea Sodium dodecyl sulphate and Polyacrylamide gel electrophoresis (Urea-SDS-PAGE) of tissue extract and mucus was separated through 12% Urea-SDS-PAGE using Silver nitrate staining.

Results: The patterns of protein bands indicated a distinct pattern of four bands with weak staining were observed in the tissue extract and two in mucus extraction and one additional band with weak staining. The protein patterns were identified by using a marker relative molecular weight of approximately 23-175KDa. Rm value of protein bands were calculated accordingly.

Conclusion: The electrophoretogram revealed that both the protein patterns of tissue and mucus extracts showed homology in protein bands with minor variations.

Key words: Arion ater, mucus, Urea SDS-PAGE, Rm value, Protein bands.

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INTRODUCTION

There is a growing interest in molluscan especially gastropod natural products or secondary metabolites. This field of research receives the attention of investigators from various fields such as biology, ecology, biochemistry, chemistry, pharmacology and biotechnology. In the industrialized countries, about 25% of all prescription drugs contain active principles that are extracted from molluscs. In traditional Indian medicine, especially Sidha medical preparations, the opercula, of gastropods are used as an ingredient to combat different diseases [1], Chandran *et al* reported the antimicrobial activity from the gill extraction of *Perna viridis*. Mucus was traditionally used medicinally from Ancient Greece to the Middle Ages internally against gastrointestinal ulcers, and in the form of syrup, to soothe a cough and it contains rich in proteins of high and low molecular weight hyaluronic acid and antioxidants[2], Kubota *et al* worked on the purification and characterization of an antibacterial factor from snail mucus. The secretion of the molluscs supposedly has a double function when applied to human skin on one hand it is claimed to stimulate the formation of collagen, elastin and dermal components. The secretions of the pedal glands of the *Arion ater* were known to contain several bioactive compounds, which are species specific.

So far, there are few reports on the protein patterns of *Arion ater* through Urea-SDS-PAGE. The present investigation has been undertaken for the study of protein patterns of tissue extract and pedal gland secretion of *Arion ater* (Black slug) through Urea-SDS-PAGE in order to understand their possible defense role against microbial infections.

MATERIALS AND METHODS

Animal materials chosen for the study:

The Slugs were collected from fields of Komatipally village, Warangal, Telangana, India. The collected animals were identified by using standard manuals.

Extraction and collection of Samples: Mucus (5 ml) was collected from roughly 50 individuals by stimulating the surface of live slugs by small plastic syringe (5 ml). The samples were stored at -20°C in a deep freezer until analysis according to Sallam *et al.*, After collecting the mucus whole body were crushed and weighed to the nearest milligram. The mucus as well as tissue extractions 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 for preparation of Urea SDS-PAGE:

The supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS β -mercaptoethanol and bromophenol blue as the tracking dye. An aliquot of 0.1ml (5mg) of the tissue extract was loaded on to the 10M-Urea separating gel directly. The lower chamber electrode buffer 1M Tris and 0.2% SDS adjusted to pH 7.8 with concentrated H₂SO₄ was used for the Anderson *et al.*, [3] and whereas upper chamber electrode buffer 0.074 M Tris, 0.1% SDS adjusted to pH 7.8 with concentrated HCl. 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 was used for Lamelli's method [4]. The current supply was terminated when the tracking dye migrated to a distance of 8 cm from the origin.

Staining Procedure with silver nitrate for Urea gel electrophoresis:

For silver staining, the gel was placed in a fixative (50% methanol, 12% acetic acid) for 1 hr with gentle shaking. Thereafter, it was submerged into 50% ethanol twice for 7 min each and washed twice with distilled water, 10 min each. The gel was treated with 0.025% Hypo for 1 min, rinse the gel in distilled water for 3 times for 1 min. then stain with AgNO₃ (200mg/100ml) containing 75 μ l formaldehyde/100ml for 20 min. rinse the gel in distilled water for 40 seconds. The gels were developed in 100 ml of sodium carbonate solution containing 50 μ l formaldehyde solutions for 5 to 10 minutes. The color formation was stopped by gradual washing in 1 to 5% acetic acid and finally stored in 5% acetic acid. The gels were analyzed by silver nitrate staining method described by Switzer *et al.*, [5]. The de staining was done by repeated washing in 1 to 5% acetic acid and finally stored in 5% acetic acid.

Standardization of protein bands:

The molecular weight standards used in comparing the variations noticed in the Urea-SDS-PAGE were of molecular weight protein standards (23 to 175 KDa) from the Bio-Rad-Chemicals company from USA.

RESULTS

The electrophoretogram obtained from tissue and mucus extract of the protein patterns of *Arion ater* and their relative mobilities are presented Figure: 1 and table: 1 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 tissue and mucus extract. Protein band comparison of various regions

with standard marker proteins related that the variation is higher in the fast moving zone "C" (mol.wt 36-23KDa) and those with middle region "B" (mol wt 58-46KDa). The pattern observed in the slow moving zone "A" (mol.wt 175-80KDa).

The electrophoretic patterns of proteins of tissue and mucus extract on Urea SDS-PAGE indicated a less number of protein bands in mucus but intensity is same with the compared to the tissue extraction. In the slow moving zone "A" (mol.wt 175-80KDa) a protein band with Rm value 0.23 (mol.wt 175KDa) showed mucus extraction. A protein band with Rm value 0.35 (mol.wt 80KDa) was observed in only tissue extract of *Arion ater* but it is not observed in mucus. The Rm value 0.44 protein band was observed in only tissue extract and 0.42 protein band observed in only mucus of middle region "B" in

between the molecular weight (58-46KDa). In the fast moving zone "C" (mol.wt 36-23KDa) the Rm value 0.60 (mol wt 36KDa) was observed in the tissue extract and the Rm value 0.58 (mol.wt 36) was observed in the mucus and the Rm value 0.62 (mol wt 23) was observed in the tissue extraction of *Arion ater* but it is not observed in the extraction of mucus.

DISCUSSION

The pattern of proteins observed in the tissue and mucus extract of slug on Urea SDS-PAGE gel indicated a distinct of four protein bands and one additional band with weak staining on the gel in slow moving zone. Therefore, the protein patterns of tissue and mucus extraction of the slug exhibited some regions are nearest similarity (Fig.1)

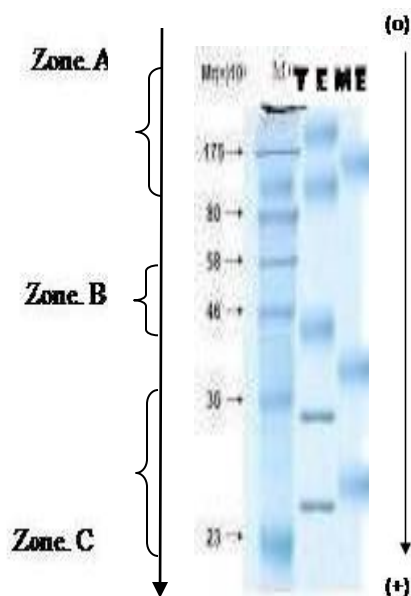


Fig. 1: Urea-SDS-PAGE Electrophoretic Patterns of Proteins of *Arion ater* tissue and mucus extract stained with Silver nitrate

Right lane indicates (M) mol.weight standards (175-23), 'A', 'B', 'C' zones
TE=Tissue extract, ME=Mucus extract: zone A= mol wt (175-80), zone "B" (58-46),
zone "C"(36-23), += indicates anode. ↓ =Direction of run, o = indicates origin.

Table1: Rm values of tissue and mucus extract of *Arion ater* (Black slug) Through Urea-SDS-PAGE.

S.No	Molecular marker standards	Tissue extract	Mucus
1	175	0.23	0.23
2	80	0.35	-
3	58	0.44	0.42
4	46	-	-
5	36	0.60	0.58
6	23	0.62	-

The presence of protein bands with identical mobility in the tissue and mucus extract indicated the similarity of proteins secreted by mucus glands (venom). Various authors have reported that the Venoms are animal secretions predominantly injected into another animal for the purposes of predation or defense, and also have important biological applications with biomedical relevance Twede *et al* [6]. In the present study Urea SDS-PAGE analysis showed the presents of bioactive compounds responsible for antibacterial activities. The presence of antibacterial compounds in the oyster *Pteria chinensis* and bivalve *Perna viridis* have been reported using the various solvent extracts [1, 7]. As the molluscan resources are rich and varied in species to species, there exist a great potential for the extraction of bioactive compounds of medicinal importance at a lower cost .

CONCLUSION

The study demonstrates the effects of tissue and mucus extract on Urea- SDS- PAGE, characterization of the protein responsible for the bioactivity. Further purification and structural elucidation of compounds are required to confirm the designation of venoms and tissue in the proposed groups. This will greatly help utilize these compounds for the prosperity and well-being of human kind. Thus, the results of the present study indicate a very strong anti microbial activity of *Arion ater*. The study strongly suggests that use the commercially available and protein rich (bactericidal) in therapeutics for the development of novel antibiotics against multiple drug resistance (MDR) pathogenic microbes. Anti peptides could be utilized as a probing tool to investigate the pharmacological potential. These characteristics emphasize the need for isolation and molecular characterization of new active anti peptides in *Arion ater* in near future.

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