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**SDS-PAGE PROFILE AND BIOCHEMICAL STUDY ON THE VENOM OF SOME  
MEDICALLY IMPORTANT SNAKES OF SINDH**

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**ABSTRACT**

Two medically important groups (families) of venomous snakes are found in Sindh province of Pakistan i.e. *elapidae* (*Naja naja* & *Common Kraits*) and *viperidae* (*Russell's Viper* & *Echis Carinatus Socherueki*). Snake venom contains many constituents and medically important nontoxic components. Approximately 90% of dry weight of most of the venom contain protein and metalloproteins material. Most toxic and biologically active components of the venom are (toxic and nontoxic) proteins. The present study revealed that the concentration of total carbohydrates was estimated, in *Russell's viper* (2.49%), *Naja naja* (1.71%), *Common Kraits* (3.34%) moreover the Protein concentration of crude venom was measured by the Lowry method and the results revealed that venom was found 56%, 74%, 89% and 52% of protein in *Naja naja*, *Kraits*, *Russell's viper* and *saw scald viper* respectively. SDS-PAGE electrophoresis technique were used by two method reducing and non-reducing method. However, through present research and detailed investigation the important biological properties of the venom was determined and concluded that the medically important and biologically active components are

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present in snake venoms of the common families of snake at Pakistan in Sindh and these results will be advantageous in the field of medicines and beneficial for curative purpose.

**Key words: Biochemical, SDS Profile, Elapidae, Viperidae Family, Sindh**

## INTRODUCTION

Major important venomous animals consist of 6 groups mostly found in sea water, hymenopterans, poisonous black and brown in color scorpions, spiders, sea snakes, and venomous terrestrial snakes species, bees and wasps. Poisonous fluids material used for catching prey, on the earth inhabiting both land and sea are recorded over 3000 species of the venomous and non-venomous snakes. Whereas, about 65 species of snakes pose serious health hazards to man in different countries of the world (1). About 500,000 venomous snake bites are reported in the world annually, resulting in about 25,000 deaths each year worldwide general mortality rate of about 5% (2) and in Pakistan 1.9 per 100,000 population estimated annual mortality rate due to snake bite (3). In South Asia, four common venomous snake species bite cases were recorded and majorly affected areas of the south Asian region countries i. e; Srilanka, Bangladesh, India and Pakistan. The common poisonous snakes found in Pakistan are *Elapidae family, Cobras, Krait, viperidae family Viper, Persian Horned Viper* (4) and the most toxic snakes belong to *elapidae and viperidae*

*family. Naja naja* (common Black cobra/karo) and *common krait (Bungarus caeruleus)* belongs to *Elapidae family* whereas *viperidae family Snakes are Russell's viper (Daboia russelii) , Saw-scaled viper (Echis carinatus Socherueki)* (5,6,7).

The purpose to design this research work was to determine the medically important and biologically active components present in snake venoms of the Pakistan at Sindh. The study be helpful for various therapeutic and pharmaceutical applications as well.

## MATERIALS & METHODS

### Venom Collection

Species under studied were identified and classified according to standard criteria. Venoms from each species of snakes were extracted, by squeezing the glands and collected in pre washed sterilized Beakers during squeezing the front fangs of snakes extended completely by opening the snake's mouth and applying frontal compression to the quatrato-articular joint. The yellowish liquid (poison) were collected and labelled (8).

### Drying of Venom

Snake venom of the selected species were dried upon desiccant (silica gel or calcium chloride) and venom were put into the vacuum desiccator, covered with a layer of gauze and sealed at room temperature. After vacuum desiccation Snake venom was turned to yellowish block sized particle crystals (9).

#### Estimation of Total Carbohydrates

Carbohydrates are the most important organic compounds such as monosaccharides. Carbohydrates from the venom of *Naja naja*, *Common Krait*, *Russell's viper* and *Saw Scaled Viper* were estimated by anthrone method against blank absorbance at 630nm (10).

#### Estimation of Total Proteins

Total Protein concentration of the crude venom of different species (*Naja naja*, *Common Krait*, *Russell's viper* and *Saw Scaled Viper*) was determined through Lowry's method was used (BSA) as standard(11).

#### Analytical SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

A slab thick gel containing 10% acrylamide was prepared from solution given in Table 1. After the completion of polymerization of lower gel, the solutions for the upper, large-pore gel were mixed in the proportion (12). The layer of water was removed from the top of the lower gel and solution for upper gel was poured over the separating gel in the presence of a plastic comb to make 10 wells. The capacity of each well is 100-200  $\mu$ l (microliter). The crude venom samples were boiled with sample buffer. Labelled gel was removed from the plates, Coomassie Brilliant Blue R-250 were used for staining and before destained with the destaining solution. It is noted that (13) the covalently bound dye adjusts the apparent the M.W based protein have a tendency to produce broader bands.

Table 1: Staining and Destaining Chemicals

De-staining solution	
Methanol	250 ml
Glacial acetic acid	70 ml
Distilled water	680 ml
10% separating gel with SDS	
Distilled water	24.4 ml
Acrylamide mix	20 ml
3M Tris-HCl buffer pH 9.5	15 ml
10% SDS (w/v)	0.6 ml
10% Ammonium per-sulfate	0.6 ml
TEMED	0.025 ml
4% Stacking gel with SDS	
Distilled water	13.6 ml
Acrylamide mix	3.4 ml
0.5M Tris-H <sub>3</sub> PO <sub>4</sub> buffer, pH 9.0	2.5 ml
10% SDS (w/v)	0.2 ml
10% Ammonium persulfate	0.2 ml
TEMED	0.025 ml

### Data Analysis

All the experiments had three replicates. Data was analyzed for one-way analysis of variance followed by Student-Newman-Keuls multiple test at 0.05 level using compare means procedure of SPSS 16.

### RESULTS

The present study revealed that the concentration of total carbohydrates shown in Table 2 that total carbohydrates of *Elapidae* family (*Naja naja* and common *Kraits*) *viperidae* family was estimated at 630nm and the high percentage of total carbohydrates was observed in *Russell's viper* (2.49%), whereas in *Naja naja* (1.71%), *Common Kraits* (3.34%) and saw scaled viper (1.9) was observed. Protein concentration of crude venom was measured by the Lowry method and the total protein in crude venom of (*Naja naja*, *Common Kraits*, *Russell's viper* and *Saw Scaled viper*) was found 56%, 74%, 89% and 52 % respectively were found. Snake venom contain large number of different molecular weight proteins and peptides affecting hemostatic system and is a rich source of original complexes that may have important applications in the field of medicine and biochemistry (13). *Viperidae* family Venoms contain a large number of variety of proteins and peptides affecting the haemostatic structure and a rich source of

original compounds that may have applications in the field of pharmaceutical and biochemistry (13). Snake venom bearing lectin-like C-type protein and perform activity as anticoagulant (15,16)

Moisture content of *Elapidae* family (*Naja naja* and *Kraits*) *viperidae* family (*Russell's viper* and *Saw Scaled Viper*) was estimated at room temperature under vacuumed condition, high percentage of moisture was observed in *Russell's viper* (77%), *Naja naja* (72%), *Kraits* (67%) and the low percentage of moisture was observed in *Saw Scaled viper* (62%).

### SDS-PAG Electrophoresis

In present study the SDS-PAG electrophoresis technique were used by two method reducing and non-reducing method results are shown in figure. Our results, based on the polyacrylamide gel electrophoresis, indicated that the crude venom of the *Elapidae* and *Viperidae* family (protein bands) are shown in figure. Venom of *Elapidae* family is more complex compared to the *Viperidae* Family.

Minton and Weinstein (1987) use SDS-PAG electrophoresis technique for venom and obtained 7-10 protein bands of proteroglyph *Elapidae* & *Hydrophidae* snake venoms that known as complex venom (17). In present study total number of protein bands was

found lower in Viperidae snakes and it was observed that low molecular weight proteins are metalloproteins and responsible for major local symptoms in snakebite, causing hemorrhage, inflammation, edema, hypotension, hypovolemia and necrosis. A condition of depressed blood pressure caused number of snake venoms in envenomed patients.

Since 1930 cobra venom was used for treating cancer and pain, as the malignant and spinal cord tumors removed by surgery but elimination were difficult and much not clear because they attack the surrounding tissue but the Disintegrins, like contortrostatin starting American copperhead (*Agkistrodon contortrix*) venom which prevent cells from sticking together resulting in blockage cell mortality rate (18, 19, 20,6).

### PROTEIN SEPARATION BY SDS PAGE REDUCING METHOD

### NON REDUCING METHOD

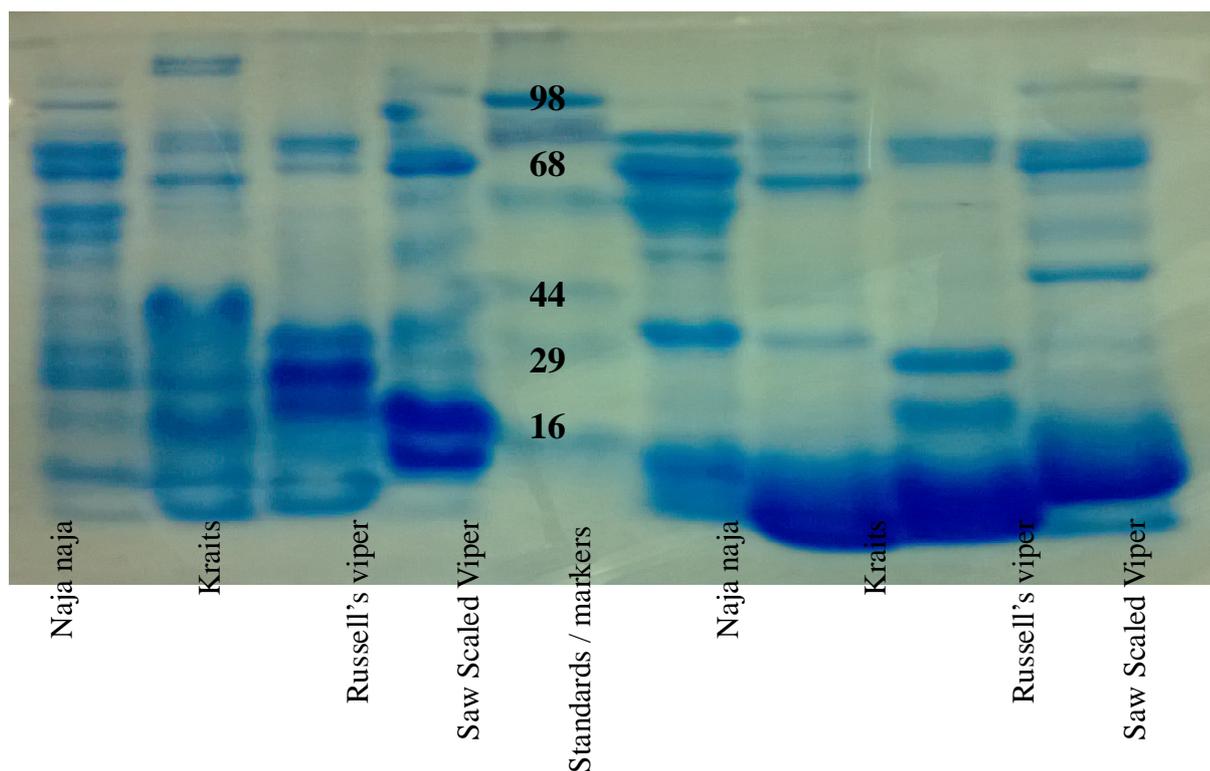


Figure 1: SDS-PAGE of crude venom of *Naja naja*, Kraits, Russell's viper and Saw Scaled viper, analyzed on 12.5% SDS-polyacrylamide gel in the presence of 1% 2-mercaptoethanol

Table 2: Biochemical parameters of Snake species

Snake Species	Total Carbohydrates (%) SD = 0.78	Total Protein (%) SD = 15.4	Moisture (%) SD = 5.84
Naja naja	2.49	56	72
Kraits	1.71	74	67
Russell's viper	3.34	89	77
Saw Scaled Viper	1.95	52	62
F statistics at df = 11	6.2	3.5	500.0
P value	0.1	0.0	0.0

## CONCLUSION

The present study revealed that the concentration of Total Carbohydrates, Protein were estimated from Common venomous snake species (*Elapidae* and *Viperidae* family) venom. Protein pattern of crude venom were (SDS-PAGE electrophoresis) determine important biological properties of the venom. Medically important and biologically active components are present in snake venoms of the common families of snake at Pakistan in Sindh and these results will be advantageous in the field of medicines and beneficial for curative purpose.

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