

# Intraspecific variation in the venom electrophoretic profile of saw-scaled viper, *Echis carinatus* of Central Punjab, Pakistan

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## Abstract

The use of Polyacrylamide gel electrophoresis (PAGE) for basic proteins may be a useful auxiliary tool for the characterization of whole snake venoms and for the taxonomic classification of snakes. The electrophoretic characterization of the basic venom proteins has emerged as an efficient method for taxonomic studies of the families Elapidae and Viperidae. The present study comprises the intra-specific protein profiling of venom of saw-scaled Viper (*Echis carinatus*) through the Sodium Dodecyl Sulfate – PAGE (SDS-PAGE) in order to understand the protein patterns of its venom. This study will also help to explain the importance of venom studies for the elucidation of taxonomic status of an important venomous snake species of Pakistan.

## INTRODUCTION

Snake venom is defined as a highly potent, biologically active mixture of chemical constituents produced or secreted by the venom gland and injected into the prey to paralyze and digest it or for defense. It has been a powerful tool to elucidate biological processes of vital importance (1). Snake venoms are a mixture of complex proteins, which have many physical and pharmacological properties. Venoms are being explored for invaluable proteins, most of which are enzymes and toxins. Many enzymes and toxins have been located, isolated and purified from different snake venoms and the mechanism by which they induce pharmacological effects has been investigated (2). Each snake species has a unique venom with different components and different amounts of toxic and nontoxic compounds. The more closely related species of snakes exhibit more similarities in their venom composition. A number of investigators all over the world have studied the various components of venoms of many groups of snakes, using the Tiselius electrophoresis, paper electrophoresis as well as PAGE. Attempts have also been made to elucidate the distinctive biological activities of the venom toxic factors (3).

The electrophoretic method is very effective not only for identification of the venom components but also for comparative biochemical studies. The reproducibility of this technique is very high and more than ten protein bands can be detected (4). Many researchers have used the

electrophoretic methods to study the protein components of venoms (5) & (6). Polyacrylamide gel has been widely utilized in the study of protein components of venoms from elapid snakes (7). Populations of some vipers have also been examined for morphology, hemipenes and electrophoretic patterns of venom proteins (8). It has been suggested that the use of electrophoresis is an auxiliary tool for the morpho-taxonomic studies along with venom identification of some species of the Elapidae and Viperidae families. (9).

Even though the relationships between species can be reflected in major toxic components of their venom, the evolution of venom is not fully understood. While studying the intra-specific variation in the components of venom it has been found that some components are conserved while others are variable. According to Glenn & Straight the geographically and ecologically isolated populations have a high potential for genetic variations. Further, the presence or absence of particular proteins in individual samples of whole venom can be attributed to differences in genes encoding these proteins, which in turn is determined by various processes that control gene frequencies in a population (1).

**Figure 1**



Saw-scaled viper *Echis carinatus* is a rough scaled snake with large eyes, wider head than neck and stocky body. The scales are heavily keeled. In Pakistan it is found in Thar and Cholistan deserts of Sindh and Punjab provinces respectively and also in Astola Island off Markan coast in Balochistan. This snake is considered to be the one of the world's most dangerous snakes because of its highly toxic venom, abundance near cultivated areas, aggressive and easily excitable temperament. Its venom is highly hemotoxic and quite potent. Serious and fatal bites by Elapidae and Viperidae snakes including the saw-scaled viper occur regularly in many villages, developed areas and some adjoining areas of major cities (1011125)

In Pakistan, studies on intra-specific differences in the venoms from these two snake species have been broadly carried out, keeping in view a few variables including age, sex and localities. Comparative biochemical and biological studies on the venoms of cobra (*Naja naja*) snakes of varying ages and size groups from different localities have also been done (14). Similarly biochemical and biological properties of the venoms from *Vipera russelii russelii* of different ages have also been investigated (15). The present study comprises the intra-specific protein profiling of the venom of saw-scaled viper (*Echis carinatus*) through the SDS-PAGE. This study will also help to explain the importance of venom studies for the elucidation of taxonomic status of a particular venomous snake species.

## **MATERIALS AND METHOD**

A total number of twelve adult saw-scaled vipers were captured from different regions of Central Punjab, Pakistan and the venom was extracted. After the completion of

experiments, eight specimens were released back in the wild with the help of the snake charmer. Four specimens were brought to the Pakistan Museum of Natural History (PMNH) and after preserving in 70% ethanol, were added to the reptile repository located in the Zoological Sciences Division (ZSD) of the Museum.

## **EXTRACTION & STORAGE OF VENOM**

The head of the each snake was seized, the posterior part was held firmly between the forefinger and the thumb of the left hand; the other fingers hold the neck against the palm of the hand. The body of the snake was then placed under the left axilla in order to prevent the animal from pulling free. The mouth of the snake was opened and its poisonous fangs were placed on the inner edge of a sterilized plastic vial. The venom was extracted by pressing the fangs very gently on the edge of the vial without using anesthetics. All the venom samples were stored at 4 Co to avoid any disruption of their natural toxic properties.

## **ASSAY & QUANTIFICATION OF VENOM PROTEINS**

Venom samples were analyzed quantitatively as well as qualitatively for proteins. Protein estimation was done by Lowry (16) using Bovine Serum Albumin as standard. Protein concentration was measured spectro-photometrically at a wavelength of 595 nm. Qualitative analysis was done by SDS-PAGE. Electrophoresis was performed as described by Laemmli (17) using a discontinuous buffer system.

## **GEL PREPARATION & ELECTROPHORESIS**

Venom samples were resolved on 10, 12.5 % SDS-polyacrylamide gels. About 10-30 µl of high and wide range protein Ladder (Sigma Cat. Nos. M-3788 & M-4038) was loaded as standard in a well and 10 µl supernatant of each of the samples was loaded with the help of Hamilton syringe in different wells. Gel was run at 30-40 volts for overnight.

## **STAINING & DESTAINING**

Gel was placed in a tray long with bromophenol blue stain and with constant agitation on shaking bath. Duration of staining was 4-6 hours. After staining, gel was shifted to destaining solution. It was destained with constant agitation until the background became transparent and protein bands become visible in the form of blue colored bands.

## **PHOTOGRAPHY & GEL DRYING**

After destaining, the gel was photographed with the help of

camera for permanent recording of results. Gel was the dried in vacuum gel drier (Heto Dry GDI, Heto Lab. Equipments. Denmark) at a temperature of 60 C for 2-3 hours.

**DETERMINATION OF MOLECULAR WEIGHTS BY SDS-PAGE**

The standard curve was plotted by calculating the Rf values of each standard protein against the log<sub>10</sub> of its molecular weight. Finding its Rf value on the standard curve and reading the log<sub>10</sub> molecular weight from the ordinate determined the molecular weight of the unknown polypeptide or protein. The antilog of this number was the molecular weight of the protein. Low (6,500 to 66,000) and high molecular weight protein markers (14,600 to 215,940 Daltons) from Sigma Chemicals and from Life Technologies were used in this study.

**RESULTS**

**PHYSICAL APPEARANCE OF VENOM**

The fresh venom of all snakes, right after extraction was fairly transparent however after some time it became translucent. The colour of all venom samples was yellow. Imperative characteristics and physical properties of venoms from some selected snake specimens are given in the table III. For convenience the readings have been given from the selected and healthier snake specimens.

**GENERAL DESCRIPTIONS OF VENOM PROTEINS**

The electrophoretic patterns of viper venom proteins are shown in figures 1 to 2. The molecular weights of the venom proteins given in the tables I & II. A maximum of 17 protein bands appeared in the one of the viper samples (fig 1). Great variations in molecular weights were observed in the individual venom samples of the species.

**Figure 2**

Table 1: Approximate molecular weights of venom proteins from the (Resolution of figure 1)

S. NO.	V1	V2	V3	V4	V5	V6	V7	V8
1	123000		123000	123000	123000	123000	123000	
2		112200	112200		112200	112200		112200
3	104700							104700
4	100000	100000	100000	100000	100000	100000		100000
5		93000		93000	93000			
6					83000	83000	83000	83000
7		72000	72000		72000		72000	
8	66000	66000	66000	66000	66000	66000	66000	66000
9	56000	56000		56000		56000	56000	56000
10	50000	50000			50000	50000	50000	50000
11	16000	16000	16000	16000	16000	16000		16000
12	14100	14100		14100	14100	14100	14100	14100
13	13000				13000	13000	13000	13000
14	11000	11000	11000	11000	11000	11000	11000	11000
15	9000	9000	9000		9000	9000	9000	9000
16	8500	8500	8500	8500	8500	8500	8500	
17		6500		6500	6500		6500	6500
18		5500	5500	5500				5500

**Figure 3**

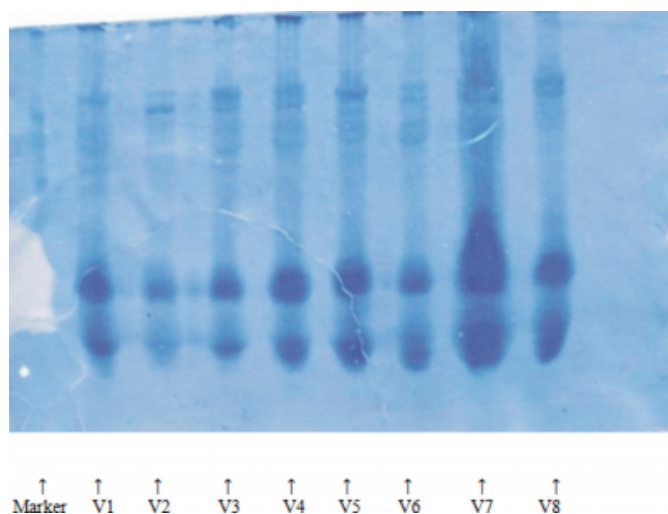
Table 2: Approximate molecular weights of the venom proteins from the (Resolution of figure 2)

Serial no	V5	V6	V7	V8	V9	V10	V11	V12
1	112200	112200	112200	112200	112200	112200		112200
2	104700							104700
3	100000	100000	100000	100000	100000	100000	100000	100000
4		92000		92000	92000			
5	85000		85000	85000	85000	85000	85000	
6	66000	66000	66000	66000	66000	66000	66000	66000
7	58000	58000		58000	58000	58000	58000	58000
8			52000	52000	52000	52000		52000
9	48000	48000	48000					48000
10	38500		38500	38500	38500	38500	38500	38500
11	14500	14500	14500	14500	14500	14500	14500	14500
12	13000				13000	13000	13000	13000
13	11000	11000	11000	11000	11000	11000	11000	11000
14	8500	8500	8500	8500	8500	8500	8500	
15	6500	6500	6500	6500	6500		6500	6500
16		5500	5500	5500		5500	5500	5500
17	4300	4300	4300	4300	4300	4300	4300	4300

With 10% gel there were only few low molecular bands in most samples. As expected, however, a larger range of low molecular weight bands appeared on 12.5 % gel (fig 2). In the entire samples, the protein bands were found almost uniformly distributed in the higher, middle and the lower portions of the gel.

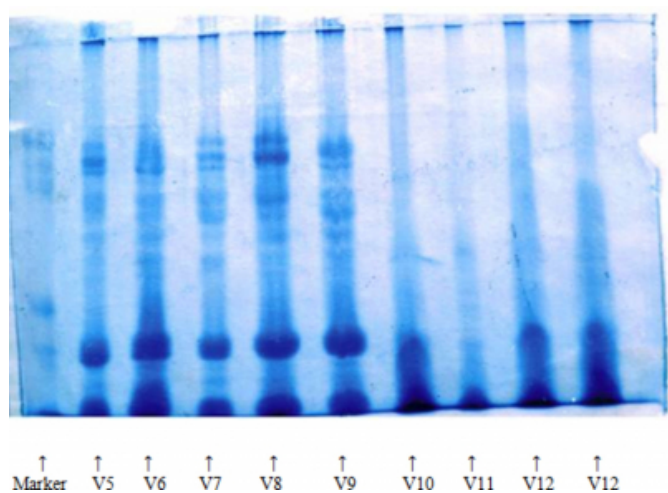
**Figure 4**

Figure 1: SDS-PAGE analysis of snake venom proteins from (10% gel).



**Figure 5**

Figure 2: SDS-PAGE analysis of snake venom proteins from (12.5% gel).



### SPECIES-SPECIFIC PROTEINS

Proteins having the molecular weights  $100,000 \pm 3000$ ,  $75000 \pm 3000$ ,  $67,000 \pm 2000$ ,  $63,000 \pm 2000$ ,  $52000 \pm 2000$ ,  $14,000 \pm 2000$ ,  $11,000 \pm 500$ ,  $5500 \pm 500$  and  $4,300 \pm 300$  were found in all the available venom samples and these proteins can be the species-specific for the given Viper species (*Echis carinatus*) (Fig 1 & 2).

An important aspect of the individual variations was the absence of an apparently species-specific protein in any one or two samples. Proteins having the molecular weights  $123,000 \pm 3000$ ,  $110,000 \pm 3000$ ,  $58000 \pm 2000$ ,  $46,000 \pm 2000$ ,  $38,500 \pm 2000$ ,  $36,000 \pm 1000$ ,  $31,000 \pm 2000$ ,  $23,000 \pm$

$500$ ,  $18,000 \pm 1000$ ,  $9,000 \pm 500$ ,  $8,500 \pm 500$ ,  $6,500 \pm 500$  and  $3,200 \pm 1000$  were found in all except one venom sample (Table I & II). Proteins having the molecular weights  $85,000 \pm 3000$ ,  $72,000 \pm 2000$ ,  $56,000 \pm 2000$ ,  $13000 \pm 1000$  and  $21,000 \pm 1000$  were also found in most (but not all) of the venom samples (Fig 1 & 2).

### INDIVIDUAL-SPECIFIC PROFILE

One protein having an approximate molecular weight  $92,000 \pm 3000$  (Table 2) was found in only three samples. Similarly another protein  $104700 \pm 4000$  was found in only two venom samples (Table 1 & 2). Such proteins appear in the venom owing to the individual differences that are common among the venoms of the snakes of all species. Regarding the classification and purification of any individual specific venom proteins for *Echis carinatus* the availability of literature is scanty.

### DISCUSSION

In this study no variables, for instance, age, season of the year, sex, diet, captivity or seasonality of snakes were not controlled. All the snakes were captured from various localities of Central Punjab province during the early monsoon of 2007, without any discrimination of gender, however all snakes encountered and captured were male. With respect to age, all studied animals were adult, though it was not possible to determine their precise age. The venom samples were collected on different days, which did not allow for providing similar diet to all specimens as a few specimens were captured just prior to the experiment.

The absence of any, otherwise most frequent, protein in any one or two samples should not deprive that protein of its probability of being species-specific along with those proteins that are found in all samples. Some examples are such proteins are  $123,000 \pm 3000$ ,  $110,000 \pm 3000$ ,  $58000 \pm 2000$ ,  $46,000 \pm 2000$ ,  $38,500 \pm 2000$ ,  $36,000 \pm 1000$ ,  $6,500 \pm 500$  and  $3,200 \pm 1000$ . These were found in all except one venom sample (Table I & II). Any such nonappearance of a protein may rather signify the diverse individual variations that usually appear as a result of the various factors affecting the composition of venoms of almost all snake species. Venom composition can vary among individuals of the same species, and even in the same litter, but variation is greater among geographically different populations. Variation in snake venom composition even in natural conditions has been associated with factors such as geographical origin, season, sex, age and diet (181519).

Extrinsic individual variations in venom composition have also been observed even under experimental conditions in which these factors are well under control (9). Such variations could be the result of intrinsic or genetic factors. According to Daltry et al, variation in venom composition results from natural selection and not from changes in diet. Venom composition may also vary over time in the same individual. The proposition that a particular protein is specific for the species is due to the fact that any such protein suggested to be specific for one species is either generally absent or is less frequent in all the samples of the other species. Regarding the classification and purification of any individual specific venom proteins for *Echis carinatus* the availability of literature is scanty.

The results of this study show great individual variations in the protein profile of the venom of *Echis carinatus*. This observation is in agreement with the other reports that individual variations exist in snake venom composition (2022232425).

While species-specific protein bands can be useful in the profiling of that species, locating the intra-specific protein bands of a venomous snake can help to establish its species status on a more reliable and definitive level. Such work may sequentially result in the identification, classification and segregation of any two venomous snake species by examining and evaluating their respective protein-banding patterns. Such protein profiling can, therefore, be an effective supplementary tool to the taxonomic studies of venomous organisms bringing authenticity to their identification and classification.

**Figure 6**

Table 3: Some physical properties of the venom from specimens used in the present study

Snake species	Sex	Approximate Age (Years)	Color of venom	Quantity of Venom Extracted (mg)	Absorbance
Viper ( <i>Echis carinatus</i> ) sample 1	Male	9-11	Dark yellow	57	0.230
Viper ( <i>Echis carinatus</i> ) sample 2	Male	10-12	Dark yellow	25	0.138
Viper ( <i>Echis carinatus</i> ) sample 3	Male	8-10	Yellow	60	0.613
Viper ( <i>Echis carinatus</i> ) sample 4	Male	9-12	Dark yellow	52	0.694
Viper ( <i>Echis carinatus</i> ) sample 5	Male	9-10	Dark yellow	63	0.520
Viper ( <i>Echis carinatus</i> ) sample 6	Male	8-10	Dark yellow	45	0.673
Viper ( <i>Echis carinatus</i> ) sample 7	Male	10-12	Dark yellow	65	1.124
Viper ( <i>Echis carinatus</i> ) sample 8	Male	9-10	Dark yellow	36	1.165
Viper ( <i>Echis carinatus</i> ) sample 9	Male	10.12	Yellow	43	1.108
Viper ( <i>Echis carinatus</i> ) sample 10	Male	9-11	Dark yellow	28	0.290
Viper ( <i>Echis carinatus</i> ) sample 11	Male	10-12	Yellow	32	0.157

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