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Short Communication

Chemical fingerprinting of Saudi Arabian snake venoms using gel filtrationchromatography.

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Abstract

Snake bite is crucial medical emergency while the success of immediate care and anti-venom therapy mainly depend on the correct identification of culprit snake. We present a simple method for identification of six snake species including *Echiscoloratus, Echispyramidum, Cerastescerastesgaspreti, Bitisarieatans, Najahaje Arabica,* and*Walterinnesiaaegyptia,* using the gel filtration chromatographic profiles of their venoms. The chromatograms of venoms from different snake species showed peculiar patterns based on the number and location of peaks.Our findings suggest that chromatographic profiles of snake venoms provide a simple and reproducible chemical fingerprinting method for quick identification of snake species.

Keywords: Snake venom, Chromatographic profiles, Elapidae, Viperidae, Identification

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Introduction

The venoms from Elapidae and Viperidae snakes are complex mixturescontaining different components such asmetalloproteinases, proteolytic enzymes, phospholipase, serine proteinase, presynaptic and postsynaptic neurotoxins, potassium channel-binding neurotoxins, cytotoxins, cardiotoxins and platelet aggregation inhibitors [1-3].Newton et al [4] have suggested that analysis of venom components can produce a unique fingerprint to be used as a valuable reference tool in taxonomic analysis asa complementary method to morphology and behavioral characterization for species identification and classification. Compositionaldifferences between snake venoms can be employed as a taxonomy signature forunambiguous species identification independently of geographic origin andmorphological characteristics [5].Recently, Calvete [6] have pointed out that use of proteomics approaches in identification of evolutionary and immunoreactivitytrends among homologous and heterologous venoms may aid in the replacement of thetraditional geographic- and phylogenetic-driven hypotheses for antivenom production strategies.

In continuation to our previous work on chemical fingerprinting of scorpion venoms [7], we performed the gel filtration chromatography of snake venoms from four species from the family Viperidae (*Echiscoloratus, Echispyramidum, Cerastescerastesgaspreti*and *Bitisarieatans*) and two species from the family Elapidae (*Najahaje Arabica and Walterinnesiaaegyptia*) and compared the chromatographic profiles for their application in species identification.

Materials and Methods

We collected six species of snakes (Fig. 1) including *Echiscoloratus, Echispyramidum, Cerastescerastesgaspreti, Bitisarieatans, Najahaje Arabica and Walterinnesiaaegyptia*from the Riyadh region of Saudi Arabia. The snakes were kept in plastic boxes and fed onmice and water *adlibitum*. The crude venom was diluted with distilled water, properly mixed, and centrifuged at 10,000 rpm at 4°C for 20 min to separate the mucus. The clear supernatant was filtered through 0.20 μm filter before chromatography.

Gelfiltration chromatography was used for venom fractionation on Superdex200 PC 3.2/30 column. Venom solution was diluted in 0.05 M sodium phosphate buffer containing 0.15 M NaCl (pH 7.0). An aliquot (25μl) of venom solution was loaded in a previously equilibrated column with the same buffer. The sample was injected using an Auto injector. The flow rate was adjusted at 0.4

mL/min and the UV range was 0.02 AUFS (absorbance unit full scale). Column operational pressure was 1.5 MPa. The elution profile was monitored at 280 nm by a UV spectrophotometer (AKTA Micro System). All the samples were run in triplicate to confirm the reproducibility of their chromatographic patterns.

Results and Discussion

All the snake species showed peculiar chromatographic profiles of their venoms depending on the location and height of the peaks (Fig. 2). The minimum numbers of peaks were observed with the venom of *Cerastescerastesgaspreti*(3 peaks) and the maximum number of peaks with the venom of *Walterinnesiaaegyptia*(7 peaks). Both the members of the genus Echis (*Echiscoloratus*and *Echispyramidum*)showed 5 peaks each (3 common peaks) whereas the venoms of *Najahaje Arabica* and *Bitisarieatans* resulted in 4 and 6 peaks respectively (Fig. 2).

The venom profile-based chemical fingerprinting clearly differentiated the two species of the family Elapidae (*Naja Arabica* and *WalterinnesiaAegyptia*) from the members of the family Viperidae. The venom profile of *Echiscoloratus* was more closely related to the venom profile of *Cerastesgaspreti* instead of *Echispyramidum* (Fig. 2).Aird [8] compared the gel filtration profiles of crude snake venoms from 38 Crotalusviridis,representing the subspecies concolor, viridis and lutosus and readily distinguished these taxa. John and Kaiser [9] conducted a comparative study of the venoms from Notechisscutatusscutatus, Notechisaterserventyi, Notechisaterhumphreysi and Notechisateraterusing gel filtration resulting in slightly different elution profiles on a Superose-12 gel filtration column.The protein profile of venoms of Elapidae was identified using electrofocusing technique; the two species could easily be differentiated whereas the differences between the two sub-species were more difficult to evidence [10]. The elution profiles of the venoms of seven Bothrops species fractionated on a Mono-Q FPLC column resulted in reproducible chromatogram-showerver there was a considerable overlap of activeproteins in different species venoms [11].

In conclusion, eachsnake's venom has a unique chromatographic profile that can be used as a fingerprint to differentiate one speciesfrom the other.Our findings suggest that gel filtration chromatography of snake venom is a simple and reproducible method for identification of snake species.

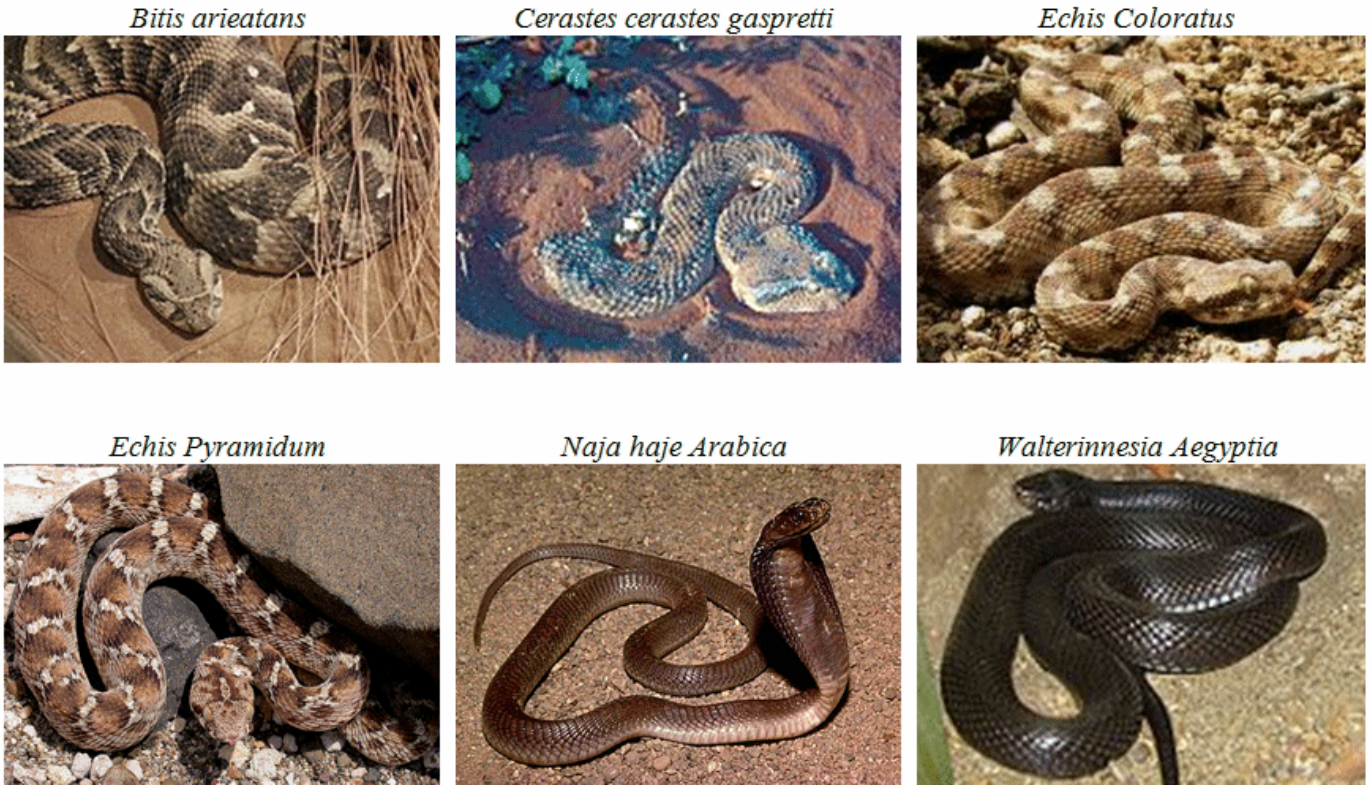

Figure 1. *Snake species used in this study.*

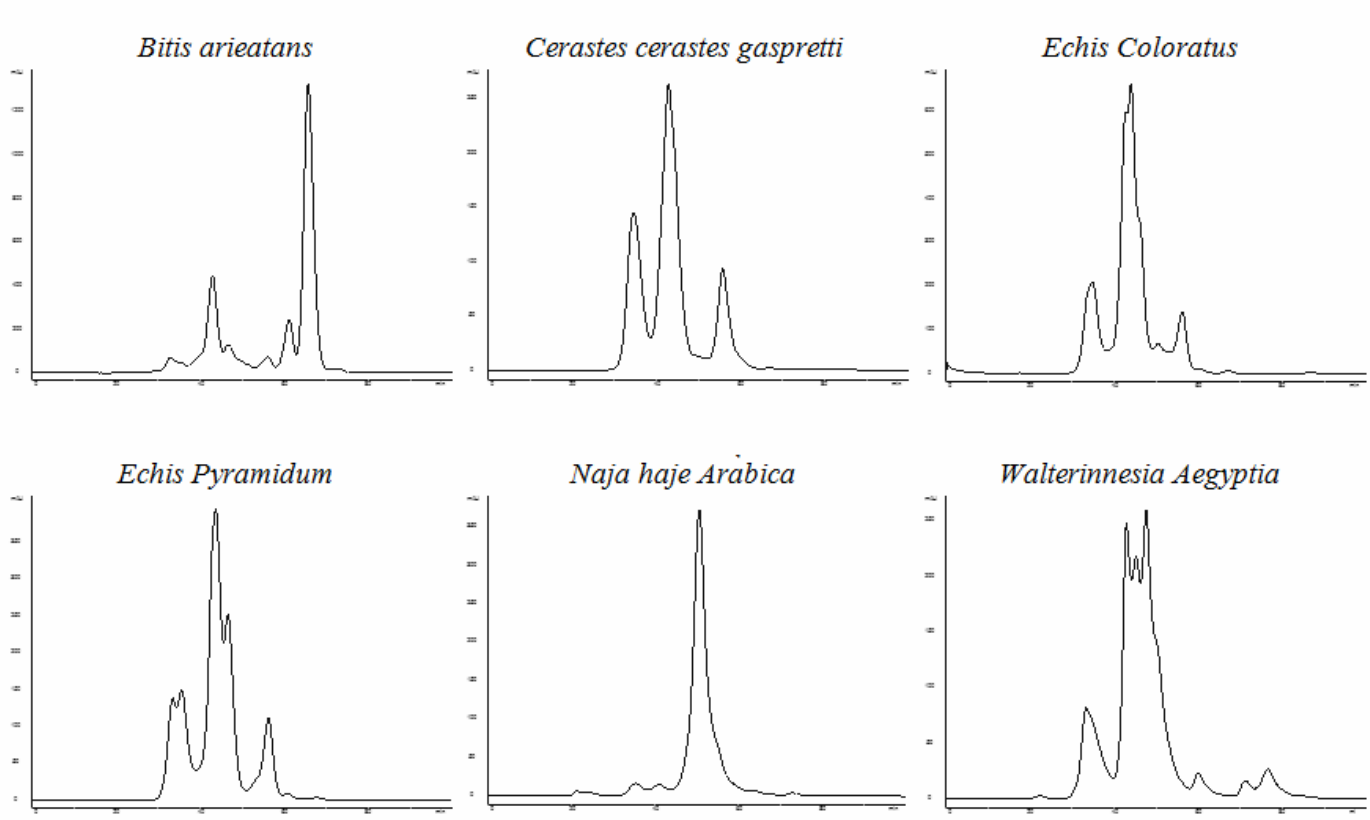

Figure 2. *Gel filtration chromatographic profiles of venoms from different snake species.*

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