RESEARCH REPORT

Elemental analysis of scorpion venoms

AbdulRahman K Al-Asmari[1,*], Faisal Kunnathodi[1], Khalid Al Saadon[2], Mohammed M Idris[1]

[1]Research Center, PSMMC, Riyadh, KSA, 11159; [2]Department of Urology, PSMMC, Riyadh, KSA, 11159

*Correspondence to: AbdulRahman Al-Asmari, Email: abdulrahman.alasmari@gmail.com

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ABSTRACT

Scorpion venom is a rich source of biomolecules, which can perturb physiological activity of the host on envenomation and may also have a therapeutic potential. Scorpion venoms produced by the columnar cells of venom gland are complex mixture of mucopolysaccharides, neurotoxic peptides and other components. This study was aimed at cataloguing the elemental composition of venoms obtained from medically important scorpions found in the Arabian peninsula. The global elemental composition of the crude venom obtained from *Androctonus bicolor, Androctonus crassicauda* and *Leiurus quinquestriatus* scorpions were estimated using ICP-MS analyzer. The study catalogued several chemical elements present in the scorpion venom using ICP-MS total quant analysis and quantitation of nine elements exclusively using appropriate standards. Fifteen chemical elements including sodium, potassium and calcium were found abundantly in the scorpion venom at PPM concentrations. Thirty six chemical elements of different mass ranges were detected in the venom at PPB level. Quantitative analysis of the venoms revealed copper to be the most abundant element in *Androctonus sp.* venom but at lower level in *Leiurus quinquestriatus* venom; whereas zinc and manganese was found at higher levels in *Leiurus sp.* venom but at lower level in *Androctonus sp.* venom. These data and the concentrations of other different elements present in the various venoms are likely to increase our understanding of the mechanisms of venom activity and their pharmacological potentials.

KEYWORDS: Scorpion, Venom, Elements, ICP-MS, *Androctonus bicolor, Androctonus crassicauda* and *Leiurus quinquestriatus*

INTRODUCTION

Scorpion venom is a rich source of biomolecules, which can perturb physiological activity of the host on envenomation and may also have a therapeutic potential (Ding et al, 2014). Being the oldest venomous species scorpion has been studied extensively for its venom. The toxic and pharmacological effect of the scorpion venom is largely due to its rich source of various small molecules and peptides. Several new drug targets have been identified from the scorpion venom through various studies. Venoms of scorpions belonging to Buthidae family are considered most toxic and medically important (Soleglad et al, 2003). *Androctonus bicolor, Androctonus crassicauda* and *Leiurus quinquestriatus* are some the major buthidae family scorpions found in the Arabian Peninsula (Al-Asmari et al, 2009).

The different constituents of crude scorpion venom are water, mucosa, oligopeptides, nucleotides, amino acids, ions, neurotransmitters, salts, low molecular weight peptides (Possani et al, 2009), metals, mucoproteins, mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamine, biogenic amines and many unidentified substances (Zlotkin et al, 1978; Possani, 1984; Müller, 1993, Gwee et al, 2002). The therapeutic effect of the scorpion venom has shown to be associated with several diseases including cancer, particularly breast cancer (Feng et al, 2008; Al-Asmari et al, 2015), leukemia (Hayden et al, 2006) and glioma (Veiseh et al, 2007; Kesavan et al, 2010).

As there is no elucidation of various elements present in the scorpion venom, we conducted this study in order to catalogue the various elements and their abundance in the scorpion venom using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The composition of the various elements and their concentrations were determined by ionization of the venom using inductively coupled plasma generated by argon gas and radio-frequency generator followed by separation and quantification using a triple quadruple mass spectrometer.

MATERIALS AND METHODS

Venom collection

Adult *Androctonus bicolor*, *Androctonus crassicauda* and *Leiurus quinquestriatus* scorpions were collected from the wild in Riyadh, Saudi Arabia. The animals were housed in the standard laboratory condition with feed and aeration. Crude venom was collected from the animals by inducing electric shock. The venoms were collected from a total of 80 animals of each category and were pooled and stored at -80°C. The experimental procedure for the venom collection was performed as per the norms of the Institutional animal care and Ethics committee, Prince Sultan Military Medical City, Riyadh, Saudi Arabia (Research protocol No. 76/2016).

Extraction of sample

The elemental composition of the venom was analyzed by mixing the venom 1 in 100 dilution with sample buffer (0.2%, v/v, nitric acid and 0.05% v/v, Triton X in water (18.2 M Ωcm^{-1})). 5 ml of the sample in buffer was prepared in replicates for the ICP-MS analysis.

ICP-MS analysis

The scanning and quantitative multi element analysis of the venom was performed using NexION 350D (Perkin Elmer, Germany) coupled with ESI-SC2DX auto sampler, cyclonic spray chamber, quartz bore injector and Meinhard concentric nebulizer for sample introduction. The operating conditions for the ICPMS analysis are as listed in the Table 1. The scanning of different elements present in the venom was assessed by performing Total Quant analysis for 80 elements using Mg, Rh and Pb as internal standards with a concentration of 10 PPB. Total of 12 readings were collected with 3 replicates per sample. The concentration of the sample was calculated at both PPB and PPM levels. Quantitative multi-element analysis of 9 elements including copper, zinc, calcium, lead, magnesium, arsenic, manganese, iron and nickel as single run was performed against the pooled standards. The standards (eight different concentrations) were prepared using the stock solutions of individual elements, which includes PPT, PPB and PPM levels (Table 2). The calibration curve was plotted using the sample buffer as blank. Using yttrium as internal standard the concentration of all the 9 elements were calculated in the pooled venom samples. All the ICPMS experiments were performed in triplicates.

Data analysis

The value obtained from the total quant analysis were analyzed for its mean and were classified in to four different categories based on concentration, such as ≥ 100 PPM, 0.1 to 100 PPM, 1–100 PPB and ≥ 1 PPB concentrations. The

Table 1. ICP-MS operating conditions.

Conditions	Results
Plasma power (W)	1400
Nebulizer gas flow (l/min)	0.93
Auxiliary Gas flow (l/min)	1.20
Plasma gas flow rate (l/min)	18
Integration	16 sweeps, 3 replicates
Scan mode	Peak hopping
Read delays	25 sec with 12 sec delay
Sample time (mins)	1 min and 16 sec
Standard mode	^{65}Cu, ^{66}Zn, ^{40}Ca, ^{207}Pb, ^{24}Mg, ^{75}As, ^{55}Mn, ^{56}Fe, ^{60}Ni Be (Intensity >2000 counts/sec)
Standard performance check	In (intensity >40000/sec U (intensity >30000/sec)
Internal Standard	Yttrium 5 µg/l
Career Solution / Running Solution	1%, v/v, Nitric acid

Table 2. List of analytes and its standards (with concentration) used for the quantitative ICP-MS analysis (concentration in ng/ml).

Analyte	Mass (amu)	S1	S2	S3	S4	S5	S6	S7	S8
Cu	62.9	5	10	20	50	100	200	400	800
Zn	65.9	5	10	20	50	100	200	400	800
Ca	43.0	500	1000	2000	5000	10000	20000	40000	80000
Pb	208.0	2	4	8	20	40	80	160	320
Mg	24.0	100	200	400	1000	2000	4000	8000	16000
As	74.9	0.05	0.1	0.2	0.5	1	2	4	8
Mn	54.9	0.01	0.02	0.04	0.1	0.2	0.4	0.8	1.6
Fe	56.9	5	10	20	50	100	200	400	800
Cd	110.9	0.02	0.04	0.08	0.2	0.4	0.8	1.6	3.2
Se	81.9	0.5	1	2	5	10	20	40	80
Ni	59.9	0.01	0.02	0.04	0.1	0.2	0.4	0.8	1.6
Y (Internal Standard)	88.9	5	5	5	5	5	5	5	5

data obtained from multi-element analysis were analyzed for their mean and ranges.

RESULTS

A total of 51 chemical elements were found to be present in the scorpion venom in different concentrations, which included 15 elements in the PPM (part per million) and 36 elements in PPB (part per billion) ranges. The elemental composition of the venoms revealed major differences in the elemental content of the venoms of *Androctonus* and *Leiurus* species. Sodium, potassium, calcium, copper, represents 96 to 98% of the total elements in all the three venoms (Table 3 and Figure 1). Based on elemental scan analysis it was found that sodium, potassium and calcium were present in the level of more than 100 PPM in all the three scorpion venoms (Table 3). In addition to the three elements copper was found in the range of more than 100 PPM exclusively in *Androctonus bicolor* venom. Magnesium, phosphorus, iron, silicon, boron, zinc, strontium, vanadium, barium and titanium were found in the range of 0.1 to 100 PPM in the venoms (Table 3 and Figure 1). In addition to these elements aluminum was found in the same range in *Androctonus bicolor* but not in the other two venoms. Fifteen elements in the range of 1–100 PPB level and 23 elements in the range of less than 1 PPB level were detected commonly in all the three venoms (Table 3). Nearly 50% of the total elements present in the *Leiurus quinquestriatus* venom was found to be sodium, which constitutes only 28 and 39% in each of the *Androctonus sp*., respectively (Figure 1). Potassium was found to be the major element found in the *Androctonus crassicauda* venom amounting to 54% of the total elemental composition (Figure 1).

Quantitative analysis of the scorpion venoms exclusively for 9 different elements showed the similar pattern as like the total scan pattern (Table 4). Copper, zinc, calcium, magnesium and Iron were found present in the venom in PPM (µg/ml) concentration, whereas the remaining four elements, lead, manganese, arsenic and nickel were found in the PPB (ng/ml) ranges. Based on the quantitative and comparative analysis it was found that copper was found maximum in *Androctonus sp.* venom in comparison to the *Leiurus quinquestriatus* venom, confirming the scan pattern. Zinc and manganese were found at higher levels in *Leiurus sp.* than in the *Androctonus sp.* venoms. It is also interesting to note that arsenic, nickel and lead were also present in the venom at detectable levels (Table 4).

DISCUSSION

Scorpion venom though well studied for its toxic and pharmacological properties at the biochemical, molecular and immunological level, it is not well understood for its chemical composition. This study has provided an insight into the elemental composition of the venoms of three medically important scorpions by using the sensitive ICP-MS approach. It is interesting to see the varying amounts of each element present in the venoms belonging to the Buthidae scorpions. A total of 51 elements were found in these three venoms at varying concentrations.

Table 3. Level of different elements present in the three scorpion venoms in different concentration ranges (n=3).

Concentration	*Androctonus bicolor*	*Androctonus crassicauda*	*Leiurus quinquestriatus*
≥ 100 PPM (µgm/ml)	Na, K, Ca, Cu	Na, K, Ca	Na, K, Ca
0.1–100 PPM (µgm/ml)	Mg, P, Fe, Si, Br, Al, Zn, Sr, V, Ba, Ti	Cu, Mg, P, Fe, Si, Br, Zn, Sr, V, Ba, Ti	Cu, Mg, P, Fe, Si, Br, Zn, Sr, V, Ba, Ti
1–100 PPB (ng/ml)	As, Mn, Cr, Fe, I, Se, Li, Y, Ni, Pb, Rb, Rh, U, Sb, Ru	As, Mn, Cr, Fe, I, Se, Li, Y, Ni, Pb, Rb, Rh, U, Sb, Ru	As, Mn, Cr, Fe, I, Se, Li, Y, Ni, Pb, Rb, Rh, U, Sb, Ru
≤ 1 PPB (ng/ml)	Ge, Ce, Sc, La, Ag, Ga, Pd, Zr, Nd, Bi, Be, Te, Tl, Sm, Dy, Gd, Er, Cs, Ho, Yb, Pr, Re, Eu	Ge, Ce, Sc, La, Ag, Ga, Pd, Zr, Nd, Bi, Be, Te, Tl, Sm, Dy, Gd, Er, Cs, Ho, Yb, Pr, Re, Eu	Ge, Ce, Sc, La, Ag, Ga, Pd, Zr, Nd, Bi, Be, Te, Tl, Sm, Dy, Gd, Er, Cs, Ho, Yb, Pr, Re, Eu

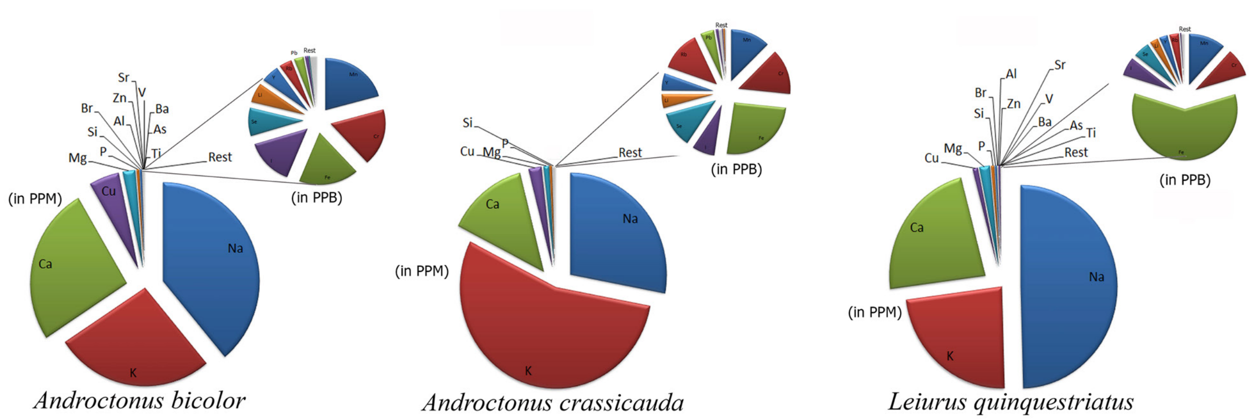

Figure 1. Pie chart distribution of various elements present in the scorpion venom. A. *Androctonus bicolor,* B. *Androctonus crassicauda* and C. *Leiurus quinquestriatus,* based on ICP-MS analysis. The distributions were presented for all the elements present in PPM and PPB ranges.

Table 4. Concentrations of the 9 elements present in the three scorpion venoms (n=3).

Sample Id	Copper (µg/ml)	Zinc (µg/ml)	Calcium (µg/ml)	Magnesium (µg/ml)	Iron (µg/ml)	Lead (ng/ml)	Manganese (ng/ml)	Arsenic (ng/ml)	Nickel (ng/ml)
Androctonus bicolor	127.6	4.6	174.9	37.6	1.4	17.9	135.8	266.7	13.2
	(± 38.9)	(±0.5)	(±8.6)	(±1.2)	(±0.5)	(±3.5)	(±27.8)	(±70.8)	(±2.3)
Androctonus crassicauda	51.6	3.1	176.0	36.5	1.3	25.7	147.4	355.4	13.3
	(±13.7)	(±0.6)	(±18.2)	(±3.6)	(±0.7)	(±4.4)	(±6.1)	(±87.0)	(±3.9)
Leiurus quinquestriatus	29.3	16.0	151.6	32.5	1.7	13.3	290.5	375.5	11.7
	(±1.9)	(±3.3)	(±6.3)	(±1.4)	(±0.6)	(±2.5)	(±26.0)	(±54.7)	(±2.8)

Based on elemental composition of the three venoms and the abundance of different element level, *Androctonus bicolor* venom has sodium as the major elemental constituent followed by calcium and potassium elements. *Androctonus crassicauda* venom has potassium as the major elemental component followed by other elements. Sodium and calcium were found maximum in the *Leiurus quinquestriatus* venom in comparison to the other venoms. Based on an unpublished semi-quantitative spectrographic analysis for various chemical components present in the Buthidae scorpion, (*Centruroides sculpturatus*), it was found that sodium (5.6%) and potassium (1.7%) were the major chemical components followed by other elements (Stahnke, 1978). Our study also revealed the similar results along with calcium and copper as the most abundant elements present in the Buthidae scorpion venoms. Distribution of elements such as sodium, potassium, copper, zinc, calcium, magnesium and iron in PPM concentrations in Buthidae scorpions might be associated with the toxicity of the venoms. Also, the difference in the elemental compositions between the venoms tested may be a reflection of the activity metalloproteinases of these venoms and their toxicity. Therefore, a comparative analysis of the elements present in the venoms and their relative toxicity would be an interesting follow up of this work, since these chemical elements may act as co-factors of the enzymes active in the venoms, influencing their toxicity.

Several metals have already been shown to be associated with scorpion metalloproteinases for various functions. For example, a high concentration of potassium element in the *Parabuthus* scorpion prevenom was shown to be involved in pain and toxicity during envenomation by enhancing depolarization and decreasing the local electrochemical gradient of cells (Inceoglu et al, 2003). Venom metalloproteinase, antarease, present in the Brazilian scorpion (*Tityus serrulatus*) was shown to penetrate intact tissue and induce pancreatitis by specially cleaving vesicle-associated membrane protein 2 (VAMP2) of the SNARE complex through the catalytic action of zinc (Ortiz et al, 2014). Similarly, calcium and zinc metal were found catalytically activating the venom metalloproteinase of *Tityus serrulatus.* Therefore, the high level of sodium, potassium, calcium, copper and zinc indicates the importance of these elements in the venom function, perhaps via interaction with metalloproteinases and other venom proteins.

CONCLUSIONS

To the best of our knowledge this is the first study reporting chemical elemental mapping of Buthidae scorpion venoms as well as their abundance. These data are likely to be of use in understanding the mechanisms of venom action and their functions in toxicology and pharmacology. A comparative analysis of the elements present in these venoms, their interaction with the venom enzymes and proteins and the relative toxicity of the venoms would be an interesting follow up of this work.

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AUTHOR CONTRIBUTIONS

AKA, KA and MMI conceived and co-ordinated the study, FK and MMI performed the ICPMS experiments and analyzed the data. AKA, FK and MMI wrote the manuscript. All the authors reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTERESTS

None declared.

ABBREVIATIONS

ICP-MS; Inductively coupled plasma – Mass spectrometry
PPB; Part per billion
PPM; Part per million

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