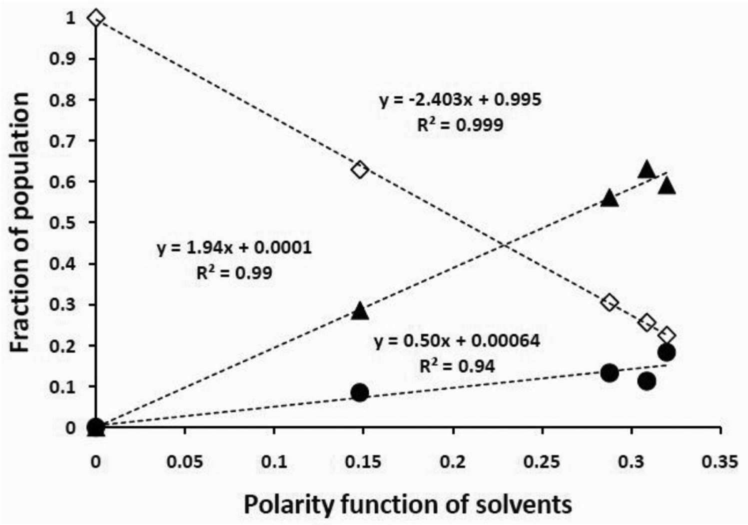

Figure 1. Dielectric constant of solvents exhibit correlation to the population distribution of three major conformers (◊, gamma; ▲, PPII; ●, α-helical) of AceProNMe. Correlation equations and R^2 values are embedded in the figure.

The present results showed a new direction to research in the area conformational studies of amino acids and peptides. The study will be extended to conformers of all the 20 naturally occurring amino acids in the proteins and inference can be drawn on the solvent effect on the conformers of the single amino acid residues. The results may provide information on the contribution of individual amino acids to the overall conformation of peptides or proteins under the environment of different dielectric constants.

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ICP-OES analysis of *Naja naja karachiensis* venom: inorganic ions for turning on and off enzymatic actions

Snake bite envenomation is resulting in high rates of mortality and morbidity all over the world. In developing countries like Pakistan, the burden of snake bite is hard to estimate due to the insufficient epidemiological data, as victims receive traditional health care instead of hospitalization. Four types of snakes cause most of envenomation. They are *Bungarus sindanus*, *Daboia russelii*, *Echis carinatus sochureki* and *Naja naja*[1]. *Naja naja karachiensis* is a subspecies of Pakistani *Naja naja*, which is responsible for large number of deaths in southern Punjab province of Pakistan. Among various complications caused by *Naja naja karachiensis* venom are bleeding wounds, haematuria, haemolysis, inflammation, bleeding gums, necrosis, coagulopathies, damage to liver, heart and kidneys[2]. Many toxic proteins in the venom have been considered to cause the detrimental effects of *Naja naja karachiensis* bites. Among them phospholipase A_2 (PLA_2), protease, alkaline phosphatase (ALPase), L-amino acid oxidase (LAAO), 5′-nucleotidase (5′-ND) and three finger toxins (3FTXs) are deadly venoms[1,3]. Snake venom additionally comprises inorganic constituents, which play a pivotal role in its toxicity. According to the literature survey, metallic ions are of prime importance in turning on and off various toxic proteins. However, their presence and quantity in venom vary from species to species. Complete knowledge about metallic and non-metallic components helped map intriguing mechanism of enzyme action and get deep insights into their severity after envenomation[4]. In this study, the inorganic components of *Naja naja karachiensis* were quantitatively measured by inductive couple plasma-optical emission spectrometry (ICP-OES) to have a complete picture of toxicity for enabling more effective treatment of snake bites in future.

Snakes (*Naja naja karachiensis*) were collected with the help of local charmers from Cholistan desert in the Punjab province of Pakistan. Venom was milked in low light atmosphere by squeezing the glands below their eyes. After collection, it was subjected to lyophilization and further stored in a refrigerator for experimental work[5].

Samples were digested in a closed microwave and pressurizing system (ultra Wave system, Milestone Srl, Sorisole, Italy). Dried lyophilized samples (100 mg) were weighed directly in digestion vials (Capitol vial, Fulton Ville, NY, USA) followed by addition of a digestion medium. Samples were digested in a mixture of 70% HNO_3 (750 μl) and 30% H_2O_2 (350 μl) at 240°C (by running a program for 1500 W microwave power) for 10 min after 15 min of ramping. Before releasing the pressure, samples were cooled to 80°C automatically. Finally samples were diluted (up to 15 ml) with Milli-Q water to confer HNO_3 in a concentration of 3.5% (ref. 6).

Full quantitative multi-elemental analysis of samples was performed with ICP-OES instrument (Optima 5300 DV, PerkinElmer, USA) equipped with a Meinhard nebulizer, cyclonic spray chamber along with auto sampler with automatic direct injection. ICP-OES was set up with the following parameters: radio frequency power, 1400 W; nebulizer flow, 0.65 l/min; auxiliary flow, 0.2 l/min; plasma flow, 15 l/min; sample flow, 1.5 ml/min. Interference free wavelengths were selected and used in axial or radial mode. All acquired data was processed using the software Winlab32 (version 3.1.0.0107, PerkinElmer). Inorganic standards (P/N 4400-ICP-MSCS, P/N4400-132565 A&B by CPI International, Amsterdam, Netherlands) were used for external calibration. Analytical accuracy was performed by certified reference material (CRM) apple leaves (NIST 1515) representing the matrix and elements of interest from the US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, USA. Data were not accepted if below the limit of detection (LOD) where LOD was three times standard deviation of at least seven blanks. Further, data were rejected if accuracy of the elements was less than 90% of the reference value[7,8].

ICP-OES experiments revealed that both metal and non-metal contents were found in the *Naja naja karachiensis* venom. Metallic elements constituted 95% of total inorganic elements while non-metals were rather limited. Among non-metals, only phosphorus was detected in reasonable amount and represented 5% of the inorganic component. As common for snake venoms, crude venom was found to possess both monovalent and divalent cations. Among monovalent cations, sodium (30%) and potassium (13%) were identified in highest concentration (43% of total inorganic contents) whereas zinc (23%), magnesium (20%), calcium (9%), manganese (0.05%) and copper (0.003%) were the most prevalent divalent cations. Molybdenum, bismuth, selenium, platinum, palladium, silver, gold, iron and cobalt were not present in detectable quantities. Table 1 gives complete details of the composition of *Naja naja karachiensis* venom.

Monovalent and divalent cations were found in *Naja naja karachiensis* venom as reported previously from Crotalidae, Viperdae and Elapidae families to counteract electrostatically charged protein molecules[9]. Literature review revealed that sodium and potassium ions comprised the highest portion of monovalent cations as reported earlier for other snake venoms. Monovalent cations do not contribute any toxic property. Nevertheless, divalent cations act as co-factors for different enzymes present in snake venom. Among divalent cations, zinc was most abundant in the Pakistani cobra compared to previously reported snakes' venom[9,10]. Zinc has been recognized to inactivate PLA_2, 5′-ND, venom nerve growth factor (vNGF) and other compounds like clavata (insecticidal compound) while it is necessary for proteolytic (acutolysin D), phosphodiesterase (PDE) and multicatalytic NADase and AT(D)Pase (AA-NADase from *Agkistrodon acutus*) activities[10–13]. All these reports strongly suggested zinc to play a pivotal role in *Naja naja karachiensis* envenomation.

Magnesium was found to be the second most abundant divalent metal in *Naja naja karachiensis* venom. It is present in higher amount than in several previously reported toxins. Magnesium has been documented to activate PLA_2, 5′-ND, LAAO, acutolysin D and for substantial binding of PDE and activation factor X (X_a) with an anticoagulant factor II[10,11,14]. On the other hand, magnesium has been proved to inhibit insecticidal activity of clavata compound[13].

Calcium was the third most important copious metal detected in this venom. It is found in greater amount when compared with *N. naja* (1000 μg/g), *N. naja atra* (1000 μg/g) and *C. horridus atricaudatus* (150 μg/g) venoms. However, it is found to be in lesser amount than in other reported venoms[9,10]. Calcium has been documented earlier to inhibit LAAO, however, it is involved in activation of PLA_2, clavata, acutolysin D, PDE and structural stability and binding of ACF II with factor X_a[10,11,13–15]. Calcium ions at 10 mM concentration are found to

Table 1. Quantitative estimation of different elements (metal and non-metal) found in *Naja naja karachiensis* venom (100 mg) via ICP-OES analysis

Elements detected	Monitored wavelength (nm)	Quantity (μg/g)	SEM	%CV
Na	589.620	4519	2	0.06
Fe	238.213	0	0	0
K	766.528	2013	5.5	0.3
Ca	315.902	1442	19	1.8
Cu	324.771	0.6	0.09	23
Co	238.902	0	0	0
Mn	257.621	6.5	0.65	14
Mg	279.090	3047	31	1.5
Zn	213.867	3473	28	1
P	213.626	718	8.5	1.6

enhance haemolytic activity posed by *Acanthaster planci* spines with 99.5% severity[16].

Manganese was detected the fourth most abundant metal ion in *Naja naja karachiensis* venom. The manganese content is not reported from most of the venoms except from the Elapidae family. Manganese is found in minute amount when compared to the other cobra venoms such as *N. naja* (200 μg/g) and *N. naja atra* (13 μg/g)[9,10]. Literature review reveals that manganese is involved in activation of 5′-ND, PDE, NADase and AT(D)Pase activities. Nevertheless, it is reported to neutralize caseinolytic activity posed by acutolysin D[11,12,15].

Copper was the least abundant metallic inorganic element detected in cobra venom. It has not been documented before in different types of venoms except in a few species of Crotalidae such as *A. acutus* (175 μg/g) and *S. milarius barbouri* (200 μg/g)[9,10]. Copper is found to activate PDE and AA-NADase. However, it diminishes haemolytic, caseinolytic (acutolysin D) and insecticidal (clavata) activity[11–13,15,16].

Phosphorus is the only non-metallic inorganic constituent detected in this cobra venom. Phosphorus content might be due to degradation of normal tissue components present in snake venom glands[4]. Phosphorus apparently lacks physiological/pathophysiological function(s) to impart snake venom toxicity.

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Screening and comparison of two edible macrofungi of *Auricularia* spp.

Auricularia is a genus comprising edible macro-fungi. It grows on fresh wood or decaying tree trunks. *Auricularia* spp., family Auriculariaceae, locally known as Uchina, is consumed as a dish widely by patients in course of therapy by local traditional healers in Manipur, India. The two species *Auricularia delicata* (Mont.) Henn. and *Auricularia polytricha* (Mont.) Sacc. are found in the hilly swampy forest. It is a group of edible type of mushrooms. Our survey indicated that Uchina (UCHI-RAT, NA-EAR in Manipuri language) is the common name of the species of *Auricularia* clubbed together as one locally. It is primarily used for treating diarrhoea, dysentery, diabetes, hypertension, constipation and liver pain in the folk medicine of Manipur[1,2], the Maiba Maibae system. *A. delicata* has been studied extensively for its artificial production, physiological properties and nutritional value[3–6]. But there has been no report on studies of antioxidant compounds of these species from Manipur. Mushrooms have been studied widely for various bioactive compounds and isolation of polysaccharides, phenolics, proteins, etc.[7]. Many other mushrooms such as *Lentula edodes*, *Grifola frondosa* and *Tricholoma lobayense* have been reported of having hepatoprotective effect against paracetamol-induced liver injury[8]. The folklore use of the above species for healing diseases of liver, literature report on high antioxidant compounds[9] and antioxidant property of chlorogenic acid which are bioavailable in humans and its antiinflammatory activities[10] prompted