Short Communication

A Comparative Study of Enzymatic Activities of *Daboia Russelii and Bungarus Caeruleus* Indian Snake Venoms

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ABSTRACT

The purpose of the study was to compare the enzymatic activities and the characteristic toxicity of the two venomous Indian snake species *Daboia russelii* and *Bungarus caeruleus*. The activity of the enzymes protease, phosphomonoesterase, phosphodiesterase, L-amino acid oxidase, hyaluronidase, 5'-nucleotidase, acetylcholinesterase and phospholipase A2 were determined in venom of *Daboia russelii* (viper) of family Viperidae and *Bungarus caeruleus* (Krait) of family Elapidae. The venom of both snakes were characterized by low levels of protease, phosphomonoesterase, phosphodiesterase, 5-nucleotidase enzyme activity. The enzyme phospholipase activity was higher in viper compared to krait, whereas the L-amino oxidase, acetylcholinesterase and hyaluronidase activities were higher in krait than in the viper venom and were statistically significant. The venom of both the snakes was toxic, but the higher amount of acetylcholinesterase, that disturbs the neurotransmission and hyaluronidase, which helps in spreading of toxin present in krait might suggest its venom being more toxic than viper.

Key Words: Acetylcholinesterase; *Bungarus caeruleus*; *Daboia russelii*; Hyaluronidase; Krait; Phospholipase A2; Viper

INTRODUCTION

Snakes are poikilothermic carnivorous reptiles, found almost everywhere on the earth's surface, with certain exceptions such as the Arctic, Antarctic and some small islands.[1] Indian subcontinent, being tropical, harbours a variety of venomous and non-venomous snakes. The major families of snakes in India are Elapidae, Viperidae and Hydrophidae.[2] There are about 40 species of Indian snakes that can cause lethal bites but it is the Big Four medically important snakes: common cobra, common krait, Russell's viper and saw-scaled viper, which are most venomous.[3] Although the environment, habitat and human activity are the main factors that determine the number of bites, saw-scaled viper claims the majority of bites.[4]

The normal function of snake venom is to immobilize the prey and to assist in digestion.[1] Snake venoms are not single toxins but a cocktail of many components, including enzymes, polynucleotide toxins, non-toxic proteins, carbohydrates, metals, lipids, free aminoacids, nucleotides and biogenic amines. These combinations confer a formidable array of toxic properties on the venom, the peptides and polypeptides being responsible for a variety of toxic properties.[2]

The purpose of the current study was to provide a comparative analysis regarding the enzymatic activities of Russell's viper (*Daboia russelii*) and common krait (*Bungarus caeruleus*) venom that would provide an overview of the kind of toxicity they confer.

MATERIALS AND METHODS

The lyophilized venoms of *Daboia russelii* and *Bungarus caeruleus* were obtained from Irula Snake Catchers Industrial Cooperative Society, Chennai, and

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were preserved at 4°C. Chemicals used in the study were procured from Sigma Aldrich, USA and Himedia Chemicals Ltd, Mumbai. 1 mg of venom was dissolved in 1 mL of 0.9% saline, centrifuged at 2000 × g for 10 min, and the protein concentration in the supernatant was estimated according to the method of Lowry et al using bovine serum albumin as a standard.[5]

Protease assay of crude venom was carried out by the method of Greenberg.[6] The 5′-nucleotidase was assayed by the method of Rowe et al.[7] Phosphomonoesterase activity was determined by the method of Bessey et al, with slight modifications.[8] The phosphodiesterase activity was determined by the method modified from Lo et al.[9] The L-amino acid oxidase enzyme was determined by the peroxidase/dye method.[10] Acetylcholinesterase activity was determined by the method proposed by Ellman et al.[11] The hyaluronidase assay of crude venom was determined by the method of Pukrittayakamee et al.[12] Phospholipase A2 assay was determined according to the acidimetric method of Tan and Tan with a little modification by using soya lecithin instead of egg yolk suspension as a source of lecithin.[9]

Statistical Analysis: The experiment was performed in triplicate, and the results were represented as the average ± standard deviation. The statistical significance between the venoms was obtained by using student's unpaired '*t*' test, and *p* values lesser than 0.05 ($p<0.05$) were regarded as significant.

RESULTS

Both the venoms (of *Daboia russelii* and *Bungarus caeruleus*) exhibited lower activity with respect to phosphomonoesterase, phosphodiesterase, 5′-nucleotidase and protease enzymes, whereas L-amino acid oxidase, hyaluronidase, acetylcholinesterase and phospholipase A2 enzymes showed higher activity. **Table 1** provides the enzymatic activities of both venoms. The values relating to protease, L-amino acid oxidase, hyaluronidase and acetylcholinesterase were statistically significant.

DISCUSSION

Enzymes are reported as among the important constituents of elapid and viperid venoms and are involved in many levels of venom action.[13] In this study, both viper and krait venoms exhibited lower activity of phosphomo-

Table 1 The Average Activities of Enzymes in Venoms of *Daboia Russelii* and *Bungarus Caeruleus*

Enzymatic Activity	Specific Activity	
	Viper	Krait
Protease*	0.78 ± 0.018	1.015
5'-Nucleotidase**	0.326 ± 0.013	0.208
Phosphomonoesterase***	0.0556 ± 0.12	0.0262 ± 0.12
Phosphodiesterase****	61 x 10^{-3} ± 1.04	66.76 x 10^{-3} ± 2.02
L-amino acid oxidase#	71 ± 5.7	214 ± 9.8
Acetylcholinesterase##	1.81 ± 9.06	3.17 ± 23.9
Phospholipase###	95.2 ± 3.07	54.53 ± 2.3
Hyaluronidase####	132 x 10^{-3} ± 6.9	338 x 10^{-3} ± 2.02

*(μM/hr/mg) 1 unit of enzyme activity is defined as the amount of enzyme required to liberate 0.02 μmole tyrosine/hr/mg
**(μM/min/mg) 1 unit of enzyme activity is defined as the amount of enzyme required to liberate 0.01 μmole of inorganic phosphate/min/mg.
***(μM/hr/mg) 1 unit of enzyme activity is defined as the amount of enzyme required to liberate 0.1 μmole of p-nitrophenol/hr/mg.
****(nM/min/mg) nanomoles of inorganic phosphate released/min/mg.
#(μ/min/mg) 1 unit (U) is defined as the amount of enzyme that catalyzes the formation of 1 μmol H_2O_2/min.
##(μM/min/mL) enzyme activity was expressed as μ mole of product released/min.
###(μM/min/mg) enzyme activity was expressed as μ moles of fatty acid released/min.
####(TRU/min/mg) 1 unit was defined as the amount of enzyme that will cause 50% turbidity reduction as 1.0 unit of international standard preparation.

noesterase, phosphodiesterase, 5′-nucleotidase and protease enzymes. The enzymes L-amino acid oxidase, acetylcholinesterase, hyaluronidase and phospholipase A2 showed higher activity. Enzymatic activities of venom from *D. russelii* species have been investigated earlier and support our findings.[14]

When the enzymatic activities of the two venoms are compared to each other, there was no significant difference with regard to protease, 5′-nucleotidase, phosphomonoesterase and phosphodiesterase. The enzymes L-amino acid oxidase, acetylcholinesterase and hyaluronidase were found to be higher in krait, while phospholipase A2 was higher in viper. In general, protease enzymes cause local oedema, blistering and necrosis.[16] The venoms of both the snakes have significantly similar activities, indicating that both are equally toxic with respect to protease.

Nucleotidase catalyses the hydrolysis of nucleotides into nucleoside and phosphate, the activity of which was almost similar in both venoms. Phosphomonoesterase and phosphodiesterase breaks the ester bonds, and their activities were almost similar in both the venoms. L-amino acid oxidase catalyzes the oxidative deamination of an L-amino acid and leads to the production of ammonia and hydrogen peroxide (H_2O_2).[15] Krait has higher activity of the enzyme when compared to that of viper, indicating that there is excess of oxidative stress caused by the venom.

Hyaluronidase helps in the spread of venom through tissues.[16] Krait venom spreads faster when compared to viper, which is indicated by the higher enzyme activity.

Phospholipase A2 inhibits electron transfer at cytochrome C level and renders mitochondrial-bound enzymes soluble. It damages red blood cells, leukocytes, platelets, skeletal muscle, vascular endothelium, peripheral nerve endings and the myoneural junction.[17] Viper has higher activity of this enzyme, which explains why it is predominantly haemotoxic in nature. Acetylcholinesterase activity is higher in krait than in viper, which is why it is neurotoxic in nature.

L-amino acid oxidase, acetylcholinesterase and hyaluronidase activities were found to be statistically significant.

CONCLUSION

This study suggests that krait venom could be more toxic than that of viper, although further studies are required to be carriout out to substantiate this observation.

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