Research Article

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DOMINANCE OF BASIC PROTEIN COMPONENTS IN THE VENOMS OF INDIAN VENOMOUS SNAKES *NAJA NAJA* AND *BUNGARUS CAERELEUS* AS CONFIRMED BY A SIMPLE YET VERSATILE PAPER ELECTROPHORESIS

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ABSTRACT:

Naja naja (Indian cobra) and Bungarus caereleus (Indian krait) are the most venomous snakes distributed throughout India belong to Elapidae family. In the present study, the venoms of both the species of snakes were subjected to a simple paper electrophoresis in different buffer systems having varying pH values to identify the venom components and compare their electrophoretic profiles. It was observed that whole venoms of both the species of snakes were resolved into the components that mainly migrated towards the negative electrode indicating most of the venom protein components are positively charged (cationic) confirming their basic nature. Therefore, it can be concluded that there is a significant similarity in the venom composition and electrophoretic profile of venoms of both the species of snakes which are closely related Indian venomous snakes of Elapidae family.

KEYWORDS:

Elapidae, Najanaja (Indian cobra), Bungaruscaereleus (Indian krait), Paper electrophoresis, Snake venom, Basic proteins.

INTRODUCTION

Snake venom comprises a highly complex mixture of proteins that requires a simple yet versatile electrophoresis technique for their characterization.[1]The more closely related species of snakes exhibit more similarities in their venom composition. Individual variability in the composition of snake venoms depends upon geographic distribution, inheritable characters, age, sex, feed, temperature and environmental conditions. It was demonstrated that elapid snake venoms contain the basic protein components.[2,3] A number of investigators have studied the protein components of snake venoms using various methods of electrophoresis on filter paper, cellulose acetate membrane and gels such as agarose, starch, polyacrylamide, etc.[4]

Electrophoretic fractionation of snake venom proteins is based on the fundamental principle that proteins and peptides when subjected to an electrical field, a force acts upon them depending on the field strength and net charge on the proteins causing migration of the protein molecules. The migration velocity of a protein molecule in an unit electrical field under defined conditions of temperature, buffer, pH and gel composition is termed as electrophoretic mobility, intrinsic property of an individual protein. Use of anticonvectional and water holding supporting media such as filter paper, cellulose acetate, starch, agarose, polyacrylamide, etc., enhances the separation by a sieving action which in turn depends on the relative size of the proteins and the pores in the supporting media. Buffer determines the direction of the electrophoretic migration to one of the electrodes.

In the present study, electrophoretic fractionation of venoms of elapid snakes *Naja naja (Indian cobra) and Bungarus caereleus (Indian krait)* was carried out to identify the components and establish their electrophoretic profiles by employing paper electrophoresis in four different buffer systems having varying pH values. It was observed that the whole

venoms of both the snakes were separated into components that mainly migrated towards the cathode indicating that most of the components are mainly positively charged (cationic) thus confirming the basic protein components in the venom. Thus, electrophoretic characterization of venom basic proteins is a simple, accessible and efficient method for identification of components, taxonomic and comparative biochemical studies of the Elapidae and Viperidae families.[5,20]

MATERIALS AND METHODS

Venoms and chemicals

Lyophilized whole venoms of *Naja naja (Indian cobra) and Bungarus caereleus (Indian krait)* were purchased from Haffkine Institute, Mumbai (India). A 10% solution of each lyophilized venom was prepared in distilled water and used for electrophoresis. All other chemicals were obtained from the commercial sources and were of analytical grade.

Paper electrophoresis

The method was carried out according to Sundermann and Sundermann.[6] Whatman No.1 filter paper strip (1.5"X2") was moistened with buffer and placed over the support frame in the electrophoretic chamber such that the ends of the filter paper were immersed in the buffer solution. 10 µl of whole venom was applied at the middle of filter paper strip. Electrophoresis was carried out at 85 volts for 14 hours at room temperature. Paper strip was removed and dried in a oven at 100 ± 5^0C for 30 minutes. Next, the paper strip was immersed in amido black stain exactly for 2 minutes and subsequently destained by 5% acetic acid in 1% methanol solution and dried at room temperature till the bands were seen.

RESULTS

Figures 1 (A – D) & 2 (A – D) shows the paper electrophoretic patterns of *Naja naja* (Indian cobra) and *Bungaru scaereleus* (Indian krait) venoms respectively at four different buffer systems of varying pH values. It was observed that most of the venom components of both the snakes were migrated to the negative electrode (cathode). The results are shown in **Table 1.**

Table 1: Paper electrophoretic profiles of *Naja naja* (Indian cobra) and *Bungarus caereleus* (Indian krait) venoms

	System	value	strength	migration
Naja naja	Acetate	6.1	0.2M	Cathodic
(Indian cobra)	Phosphate	6.2	0.2M	Cathodic
	Barbital	8.6	0.2M	Cathodic
	TrisHCl	8.8	0.2M	Cathodic
Bungarus	Acetate	6.1	0.2M	Cathodic
caereleus	Phosphate	6.2	0.2M	Cathodic
(Indian krait)	Barbital	8.6	0.2M	Cathodic
	TrisHCl	8.8	0.2M	Cathodic

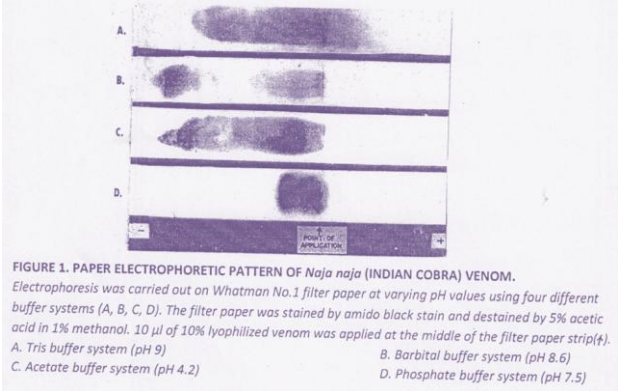

FIGURE 1. PAPER ELECTROPHORETIC PATTERN OF *Naja naja* (INDIAN COBRA) VENOM.
Electrophoresis was carried out on Whatman No.1 filter paper at varying pH values using four different buffer systems (A, B, C, D). The filter paper was stained by amido black stain and destained by 5% acetic acid in 1% methanol. 10 µl of 10% lyophilized venom was applied at the middle of the filter paper strip(†).
A. Tris buffer system (pH 9) *B. Barbital buffer system (pH 8.6)*
C. Acetate buffer system (pH 4.2) *D. Phosphate buffer system (pH 7.5)*

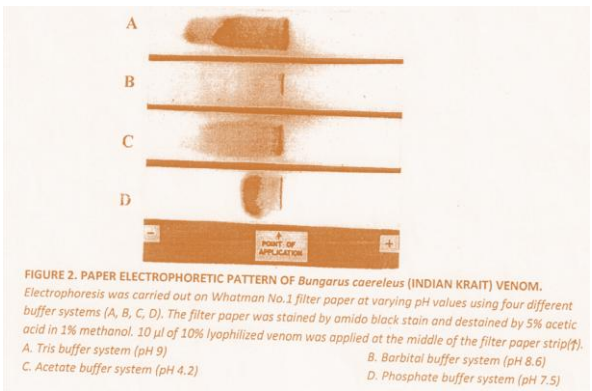

FIGURE 2. PAPER ELECTROPHORETIC PATTERN OF *Bungarus caereleus* (INDIAN KRAIT) VENOM.
Electrophoresis was carried out on Whatman No.1 filter paper at varying pH values using four different buffer systems (A, B, C, D). The filter paper was stained by amido black stain and destained by 5% acetic acid in 1% methanol. 10 µl of 10% lyophilized venom was applied at the middle of the filter paper strip(†).
A. Tris buffer system (pH 9) *B. Barbital buffer system (pH 8.6)*
C. Acetate buffer system (pH 4.2) *D. Phosphate buffer system (pH 7.5)*

DISCUSSION

Snake venom is a natural biological resource consisting of a mixture of proteins and peptides of low molecular weight having specific chemical and biological activities. Protein and peptides make up to 90-95 percent of the dry weight of the venom. Subsequently, a vast array of peptide toxins have evolved particularly for prey capture and self defence. Snake venom contains several neurotoxins, cardiotoxins, cytotoxins, nerve growth factors, lectins, hemorrhagins, disintegrins and a number of different enzymes. [7,8,9]

In previous studies, many researchers have employed various methods of electrophoresis to study venom protein components of many species of snakes. **Neumann and Habermann [10]** found two protein fractions in electrophoregram profile of snake venom using paper electrophoresis at pH 7.0 and venom components acting on frog sortorius muscle and rat diaphragm moved rapidly towards the cathode. **Riberio, L. P et al [11]** studied paper electrophoresis of snake venoms and reported the presence of large amounts of proteins in snake venom.

Master, R. W. P., Rao, S. S.[12, 13, 14] and Neelin, J. M [15] have identified enzymes and toxins in the venoms of Indian cobra, Russell's viper, krait and saw scaled viper after starch gel electrophoresis and found that Indian krait venom contain 3-4 neurotoxins. **Mohamed, AH et al and Chinzei**[16, 17] separated the myonecrotic factor from the venoms of *Naja nigricollis* (Spitting cobra) and *Trimesurus flavoviridis* (Habu) by starch gel electrophoresis into three peaks by electrophoresis.

Meldrum, B. S. [18] isolated component(s) by electrophoresis from the venom of Indian cobra *(Naja naja),* which possessed most of the toxicity of the original venom and also depolarized the frog sortorius muscle. On the other hand, **Su, Chang and Lee [19]** isolated purified neurotoxin electrophoretically from the venom of Formosan cobra *(Naja naja atra).*

The comparative studies of snake venoms using polyacrylamide gel electrophoresis showed quantitatively and qualitatively the commonality of protein and polypeptide components in the venoms of various snake species. Individual variability in the venom composition depends upon geographic distribution, age, sex, feed, temperature and environmental conditions. The differences between species and similarities between related species indicated that the protein composition of the venom is highly complex **(Soares, A. M *et al*., [3] Connolley *et al*., [20] Marshall, T and William, K.M). [21]**

Vijeyan, J et al [1] and Feroze, A et al [4] characterized the protein patterns from many elapid, viperid and Malaysian snake venoms were characterized by using polyacrylamide gel electrophoresis and two dimensional electrophoresis on the basis of isoelectric pH and molecular weight and proposed the more closely related species of snakes exhibit more similarities in their venom composition and large differences in the venom profile of each species. **Barber, C. M *et al* [2]** reported that venom is composed of proteins that mainly migrate towards the negative electrode at pH 8.6 indicating that they have positively charged proteins.

In the present study, results of the electrophoresis of *Naja naja* (Indian cobra) *and Bungarus caereleus* (Indian krait) venoms by using a paper electrophoresis at four different pH values showed a similar protein electrophoretic patterns indicating that the venoms of both the species of snakes are composed of positively charged or basic proteins that mainly migrated towards the cathode. The results of our study are in accordance with the studies reported by that of **Feroze, A et al., Vijeyan, J et al and Barber CM *et al.*** Therefore, it can be concluded that there is a significant similarity in the venom composition and electrophoretic profiles of venoms of of *Naja naja* (Indian cobra) and *Bungarus caereleus* (Indian krait) which are closely related venomous snakes belonging to Elapidae family potentially dangerous to humans responsible for many fatalities.

REFERENCES

1. Vijeyan, J., Shin Yee, L., Ponnudurai, G., Ambu, S. and Ibrahim, S., 2010. Protein profile analysis of Malaysian snake venoms by 2-Dimensional electrophoresis. J. Venom. Anim. Toxin incl. Trop. Dis. 16(4).
2. Barber, C. M., Madaras, F., Turubull, R. K., Morky, T., Dunstan, N., Allen, L., Kuchel, T., Mistschin, P and Hodgson, W. C.,2014. Comparative studies of the venom of a new Taipan species *Oxyuranus temporalis* with other members of its genus.Toxins. 6, 1979-95.
3. Soares, A. M., Anzaloni, I. L. H., Fontes, H. R. M., Da Silva, R. J and Giglio, J. R., 1998. Polyacrylamide Gel Electrophoresis as a tool for the taxonomic identification of snakes for the Elapidae and Viperidae families. J. Venom. Anim. Toxins, 4(2), 137-42,
4. Feroze, A., Malik, S and Qureshi, J., 2008. Intraspecific variation in the venom electrophoretic profile of saw scaled viper (*Echis carinatus)* of Central Punjab, Pakistan. The Internet J. Vet.Med. 6 (2), 7372.
5. Arikan, H., Gocmen, B., Kumlutas, Y., Alpagut-Keskin, N., Ilgaz, C., Yldiz, M. Z.2008. Electrophoretic characterization of venom samples obtained from various Anatolian snakes (Serpentenes: Colubridae, Viperidae and Elapidae). NW. J. Zool. 4(1), 16-28.
6. Sunderman and Sunderman., 1964. Filter paper electrophoresis of serum proteins, In: Clinical Lab diagnosis; Levinson et al (Eds), J.B. Lippincott Corp., Philadelphia, 506.
7. Sonia, S and Reema, G., 2013. Therapeutic potential of snake venoms.Int. Res. J. Pharm. 4 (11).
8. Tu, A.T., 1996. Over view of snake venom chemistry. In: Natural toxins-2, 391, Singh, B (Ed). Plenum Publishing Corp. New York. 37-62.
9. Devi, A., 1968. The protein and non-protein constituents of snake venoms: Venomous animals and their venoms. Vol. II. Venomous vertebrates. Bucherl, W., Buckley, E.E. and Deulofen, V (Eds). Academic Press, New York, 119-160.
10. Neumann, W and Hebermann, E., 1952. Zuv papier elektrophoreto*schen fractonierg tierierischegifte nature wissenschaften*. 39, 286-87.
11. Riberio, L. P., Mitidieri, E., Villella, G. G., 1955. Paper electrophoretic and enzymatic studies on blood, serum, venom and liver *of Bothrops jararaca*. Biochemical laboratory, Instituto Oswaldo Cruz, Rio de Janeiro, 53(2, 3, e 4).
12. Master, R.W.P., Rao, S. S., 1959. Identification of choline esterase in cobra venom after electrophoretic separation on starch gel. Curr. Sci. 28, 112-113.
13. Master, R.W.P., Rao, S. S. 1961. Identification of enzymes and toxins in the venoms of Indian cobra and Russell's viper after starch gel electrophoresis. J. Biol. Chem. 236 (7), 1986-1990.
14. Master, R.W.P., Rao, S. S., 1963. Starch gel electrophoresis of venoms of Indian krait and saw scaled viper and identification of enzymes and toxins. Biochim.Biophys.Acta. 71, 416-421.
15. Neelin, J.M., 1963. Starch gel electrophoresis of cobra and rattle snake venoms. Canad. J. Biochem. Physio.41, 11073-78.
16. Mohamed, A. H., Kamel, A., Sehin, R and Hani-Ayobe, N., 1972. Starch gel electrophoresis of *Naja nigricollis* venom. Enzymatic and toxicity studies. 10, 7-13.
17. Chinzei, H., 1979. Fractionation of myonecrotic factor isolated from the venom of Habu *(Trimeresurus flavoviridis)*. Jpn.J. Med. Sci. Biol. 32, 117.
18. Meldrum, B. S., 1965a. Actions of whole and fractionated Indian cobra *(Naja naja)* venom on skeletal muscle. Br. J. Pharmac. Chemother., 25, 197-205.
19. Su, C., Chang, C. C., Lee, C Y., 1966. Pharmacological properties of the neurotoxin isolated from cobra venom. Toxicon (in the press).
20. Connolley-Mendeza, C. E., Bhatti, T and Bhatti, A. R., 1989. Electrophoretic characterization of elapid, viperid and crotalid venoms. Defence Research Establishment, Suffield Roeston Alberta, Canada, 1280.
21. Marshall, T and William, K. M., 1994. Analysis of snake venoms by SDS-PAGE and 2-Dimensional Electrophoresis. Appl. Theor. Electrophor, 4(1), 25-31.

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