Effect of pH on venom of Bungarus caeruleus

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***Abstract*: Snakes have always fascinated human beings. The enzymes are present in venom and may be toxic, non toxic or enzymes with on known biological activities. The content of the enzyme may vary from venom to venom or even species to species. Snake venom has always attracted the attention of pharmacologists because of high catalytic properties. The enzymes present in the venoms are also thermally more stable. In the present study, the effect of hydrogen ion concentration has been observed on venom of Bungarus caeruleus. Two characters of venom have been observed namely toxicity by intravenous route of inoculation and immunogenicity by immunodiffusion in gel. The toxicity test has been conducted after exposing the venom to various hydrogen ion concentrations after fixed intervals of time. The results were noted in terms of lethal dose 50. To see the immunogenicity, the precipitation lines formed after keeping the venom in solutions of different pH, were observed by gel diffusion. The results have indicated that the venom is most stable at pH 7.0.**

***Keywords*: Hydrogen ion concentration, enzyme, pH. Bungarus caeruleus, immunoelectrophoresis**

Introduction

Snakes have always fascinated human beings. They are one of those creatures, which invoke both positive as well as negative responses when one hears a hissing/shrill sound or even mention of the word 'snake'. The venom of snakes contains mainly proteins and non protein components. The proteins found in snake venom is a mixture of enzymatic and non enzymatic components. The enzymes are present in all of the snake venoms and may vary from venom to venom. Snake venom serves as an attractive model to the biologists, chemists and scientists working on enzymes. This is due to their high catalytic properties and thermal stability.
Vipera russele and Bungarus caeruleus are the most common snakes found which are causing envenomation, In vitro screening and evaluation of antivenom
phytochemicals from Azima tetracantha Lam.
leaves against Bungarus caeruleus and Vipera russelli
Bhavya Janardhan1, Vineetha M Shrikanth1, Kiran K Mirajkar2 and Sunil S More1*.
The treatment is possible by injecting antivenom derived from horse serum. Some of the snake bite victims may face the anaphylactic reactions due to this. The animal in which the antivenom serum is raised also faces local reactions. It will be useful if one could have a procedure to reduce the pain and suffering to the animal. The present study is first method to reduce the toxicity of the venom but retaining it's immunogenicity.

The Indian common krait belongs to elapidae family and it's venom has hyluronidase, NAD-nucleosidase, phospholipase, acetylcholinesterase and neurotoxins.Many scintiests have worked to observe the effect of hydrogen ion on venoms of different origin. The experiments conducted by Lee etal in 1962 have shown that the venom of elapids is fairly stable at acidic pH but proteolytic and hemolytic activities reduce. In 1966 Brown also observed the effect of hydrogen ions on the venom of *Crotalus atrox* and similar results were obtained. Most of the research work with varying concentration of hydrogen ions have shown that snake venom exhibits a broad pH profile with optimum pH 7.5. The venom can withstand a wide range of variation in hydrogen ion concentration from pH 6.0 to 8.5 (David And Anderson,1981).Masatoshi et al 2008, observed that the proteinases present in venom of Agkistrodon halys blomhoffil have the values of 10.5, 9.8 and 8.9. These. proteinases were activated by the calcium and magnesium divalent ions, but inhibited by other divalent ions.

Materials And Methods

Material
1) Venom: The common krait (B. caeruleus) venom used in this study was obtained from Central Research Institute Kasauli. The venom was dissolved in sterile normal saline. It was centrifuged at 1500 rpm for 10 minutes and then passed through membrane filter of pore size 0.22 micron. The sterile solution was then used in the experiments.

2) The buffers of different pH (acetate buffer of pH 5.0, Phosphate buffer of pH 6.0, 7.0, 8.0, Barbitone buffer of pH 9.0) were prepared according to Cruichshank, 1975. The pH of all the buffers was checked accurately with pH meter.

3) Laboratory mice of 18-20 gm were taken as experimental animal. They were obtained from random breeding in a closed colony.

Methodology

Experimental Method was followed. The experiments were conducted in MVGU under the guidance of Dr. Mukesh Sharma. To see the effect of hydrogen ions the venon of *Bungarus caeruleus* was mixed with buffers of observe pH 5, 6, 7, 8, 9, in 1:1 proportion separately and kept at +4°c for 28 days. The aliquots of solution were taken after every seven days interval and checked for toxicity in vivo in albino mice by intravenous route of inoculation.

The immunological components were observed by immunodiffusion in gel by method. For this gel diffusion was done (Weir,1967 and Chase, 1971); followed by immunoelectrophoresis (Hames and Rickwood, 1985) The results were noted by observing precipitation lines.

Results

Table 1. Toxicity results observed in vivo in mice

Interval after start (days)	Visible change (if any)	Venom control Solution in Normal saline	Toxicity LD_{50} of common Krait venom in buffer solution of pH				
			5.0	6.0	7.0	8.0	9.0
0	No	1.6384	1.6384	1.6384	1.6384	1.6384	1.6384
7	No	1.6384	1.7540	1.7540	1.7540	1.7804	1.8910
14	No	1.7660	1.8910	1.8820	1.7804	1.8820	1.9930
21	No	1.7810	2.2900	1.9780	1.7804	1.9780	2.2070
28	Slight turbidity	1.8130	3.5960	2.0480	1.8910	2.5600	3.5960

It can be seen from the table that the common krait venom is most stable at pH 7.0. There was negligible change in the toxicity of venom at pH7.0 and +4°c of temperature even after storing for 28 days. Whereas toxicity of venom is affected on acidic and alkaline sides. There was not much change in immunogenicity also as can be observed in the table given below.

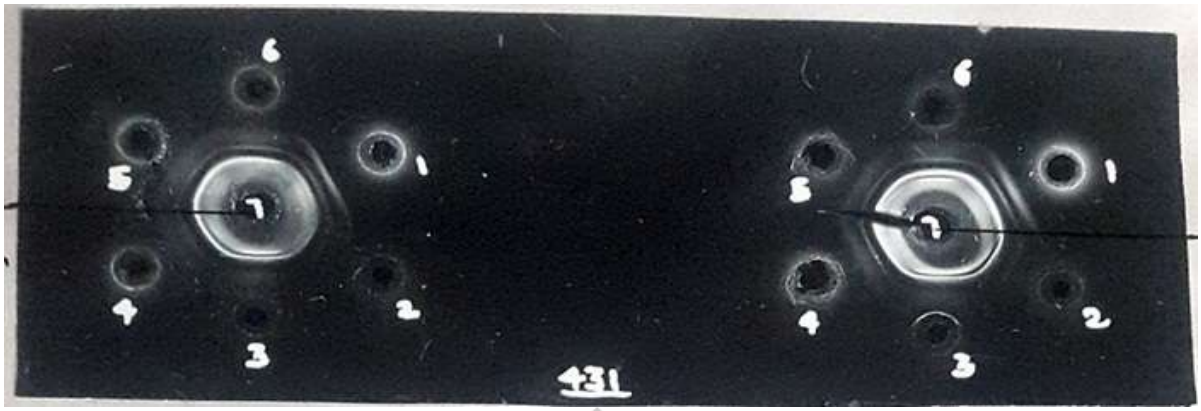
Table 2. Results of imunoelectrophoresis

Immunogenicity	Normal Saline	(Number of components retained at pH) 5.0	6.0	7.0	8.0	9.0
Lines of precipitation	7	6	6	7	7	7

Table 3 is showing the results in aggregate form so as to facilitate the comparison

		pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0
Toxicity%	Retained	50.42	88.52	100	70.82	50.42
	Lost	49.58	11.58	0	29.18	49.58
Immunogenicity%	Retained	85.71	85.71	100	100	100
	Lost	14.29	14.29	0	0	0

Effect of pH on venom of Bungarrus caeruleus (Immunodiffusion)



1. Untreated common Krait venom
2. Common Krait venom kept at pH 5.0
3. Common Krait venom kept at pH 6.0
4. . Common Krait venom kept at pH 7.0
5. Common Krait venom kept at pH 8.0
6. Common Krait venom kept at pH 9.0

Discussion

The snake venom is mainly comprised of enzymatic proteins. The common krait venom is very stable at pH 7.0. There has been no change in toxicity of the venom when stored at pH 7.0. But the precipitation lines were lost at pH 5.0 and 6.0. There has been loss of toxicity also at pH 5.0 up to 49.58%. At pH 9.0 the toxicity has been lost upto 49.58% as is observed at pH5.0, but immunogenicity has been retained.

The venom can be stored at pH 7.0 as neither toxicity nor immunogenicity is lost at this pH. On alkaline side of pH scale the venom is losing it's toxicity. Same effect can be observed on the acidic side. Antisera raised in animals is the only specific treatment available to treat snake bite victims. Antivenom sera can reverse the effects of envenomation. The present study may indicate towards the choice of a method to reduce the toxicity of venom meanwhile retaining immunogenicity.

Conclusion

Snake bite is a major problem in the areas where farming is main occupation, but it is one of the neglected areas of tropical diseases which affect people worldwide.

If we want to reduce the toxicity while retaining immunogenicity pH 9.0 and 8.0 could be a better option. The basic unit of protein is amino acid and the change in pH of a solution will affect the interactions of substrate by altering shape of the protein. The enzymatic components of the venom are affected by changes in pH because each enzyme has an optimum pH. The shape of enzyme as well as properties of the enzyme change, so that either the substrate cannot bind to the active site or it can not undergo catalysis. This may be due to the blockage of active sites that help in a reaction.

The reduction in toxicity as well as immunogenicity on changing the hydrogen ion concentration means that the structure of the enzyme or toxins present in venom has also changed. On the pH 5.0 (acidic), toxicity is retained upto 50.42% with 85.71% of immunogenic components. At pH 9.0, toxicity is retained upto 50.42% with 100% retention of immunogenic components. Only at pH 7.0 the toxicity and immunogenicity were retained upto 100% indicating that this pH is most suitable to keep the venom for prolonged storage in labs.