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*RESEARCH ARTICLE*

# EFFECT OF TEMPERATURE ON VENOM OF *BUNGARUS CAERULEUS*(INDIAN COMMON KRAIT).

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## *Manuscript Info*

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## *Abstract*

The snakes are present all over the world. They have a well-developed venom apparatus tocatch a prey. The venom is mainly composed of proteins and enzymes. Any physical or chemical gradient that influences the function or structure of proteins by denaturing its shape or by rearrangement of the functional group, brings total change in the action of the enzymatic protein. The catalytic activity of the enzyme is hindered or stopped at high temperature. As the Indian common krait venom is very lethal and the antivenomagainst it is raised in animals who in return have to suffer for serving humanity because the local reactions at the site of inoculation are found. In the present study, the venom is subjected to various temperature gradients and the effect on reduction of toxicity while retaining immunogenecity is observed by invivo in mice and in vitro by gel diffusion. It was found that the venom turns totally non-toxic at 22°C and 37°C when stored for 28 days, but immunogenicity of the venom is reduced too. At temperature 0°C, 4°C, 8°C, the venom retained both toxicity and immunogenicity even if stored for 28 days.



## Introduction:-

The snakes are found everywhere on the earth with exception of Antarctic region. Snakes have evolved from lizards and slowly evolved to their present form with well-developed venom apparatus(Kochva,1987,Heiseetal,1995 and Fry et al, 2004). Most of the advanced snake species are venomous. Indian common krait or ***Bungaruscaeruleus*** which belongs to family elapidae is also a poisonous snake with neurotoxic venom. Generally the venom apparatus is used by the snakes to catch a prey.The venom is produced in specialized glands which are modified salivary glands. It is mainly composed of enzymatic proteins and is a mixture of proteins, peptides (90-95%) with amino acids, nucleotides, lipids, carbohydrates and metallic elements bound to proteins (5%).

The present study is an attempt to observe the effect of temperature on venom that is mainly made up of proteins. In the previous research paper author has attempted to observe the effect of pH on venom and found that the venom was most stable at pH 7 (Dhir Anju,2016). The enzymatic proteins present in venom are affected by temperature variations too.Many times the enzymes get denatured or lose their potency at high temperature. The catalytic activity of the enzyme is hindered or stopped at high temperature. The venom of *Crotalusadamanteus* gets inactivated when stored at a temperature between -5$^{o}$ and -60$^{o}$C. The pH of mediumtoo influences the rate of inactivation along with temperature(Curtiet al,1968).Not only the temperature variations but also the duration of time has destructive effect on the lethal property of the venom (Winter et al, 2007).

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Any physical or chemical gradient that influences the function or structure of protein by denaturing its shape or by rearrangement of the functional group may bring total change in the action of the enzymatic protein. As the Indian common krait venom is very lethal and the antivenom against it is raised in animals, the present study is another attempt to reduce the toxicity of venom while retaining the immunogenicity of the venom, so that when it is inoculated in animals to raise antisera/antivenom, it does not bring discomfort to the animal. Moreover it can also suggest a temperature at which it can stay potent for a longer time. The knowledge about the action of temperature on venom of *Bungaruscaeruleus* can further help in characterization of this particular venom.

**Aim and Objective:-**

- The present study is an attempt to observe the effect of temperature on the toxicity and immunogenicity of the venom of *Bungaruscaeruleus* (Indian common krait).
- This may further help to select the temperature required for storing the venom in different regions of the earth having different temperatures.
- The study can also help in reducing the toxicity but retaining immunogenecity of venom by exposing to various temperatures so that the lethality of the venom is reduced for the experimental animal in which the antisera is raised.

## Materials and Methods:-

1) Venom:- The Indian common krait or *B.caeruleus*venom used in this study was obtained from Central Research Institute Kasauli& the normal toxicity of this venom was checked before starting these experiments.
2) Slides- Glass slides of 20 ×5 cm were used for immunodiffusion tests.
3) Sterile vials of 5 ml. capacity were used to keep the venom at different temperature.

Experimental method was used in the present study. Throughout experiments, the toxicity was checked by inoculating aliquots from different vials under test, intravenously in mice after fixed intervals of time. $LD_{50}$ was calculated according to Reed and Muench (Reed &Muench, 1938)

For immunodiffusion test gel- diffusion method was used, followed by staining of slides.
To observe the effect of temperature on venom, different sterile vials having equal quantity of venom in solution form, were kept at 0°C, 4°C, 8°C, 22°C and 37°C for 28 days, and the temperature was monitored from time to time.

## Results:-

The results of toxicity and immunogenicity of venom showed not much change by storing at $0^{o}$ C, $4^{o}$ C and $8^{o}$ C. The toxicity was lost/(declined) at $22^{o}$C and $37^{o}$C. The immunogenicity test shows that one antigenic component is lost at $22^{o}$C and $37^{o}$C.

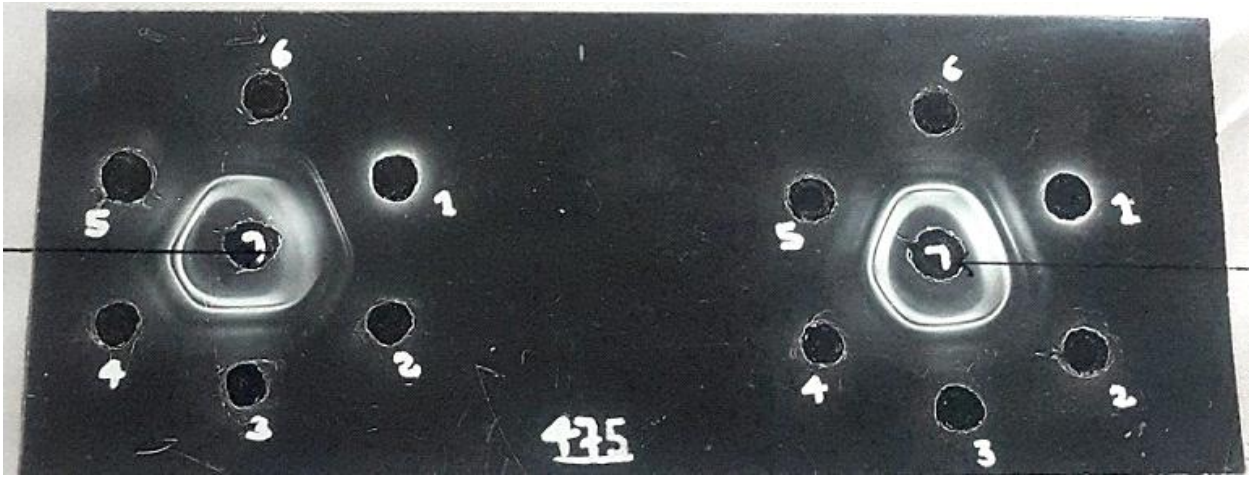


**Figure -** Effect of temperature on immunogenicityof *Bungaruscaeruleus*or Indian common krait

Venom

1- Untreated venom 2- Venom kept at $0^{o}$ C 3- Venom kept at $4^{o}$ C
4- Venom kept at $8^{o}$C5-Venom kept at$22^{o}$ C 6-Venom kept at $37^{o}$ C

**Table 1:** Effect of temperature on venom *ofBungaruscaeruleus*or Indian common krait

| Interval after start (days) | Visible change if any | $LD_{50}$ of solution of venom exposed to | | | | |
|---|---|---|---|---|---|---|
| | | $0^{0}$ | $4^{0}$ | $8^{0}$ | $22^{0}$ | $37^{0}$ |
| 0 | No change | 1.6384 | 1.6384 | 1.6384 | 1.6384 | 1.6384 |
| 7 | No change | 1.6384 | 1.6384 | 1.6384 | 2.048 | 2.832 |
| 14 | No change | 1.6384 | 1.6384 | 1.6384 | 2.345 | 2.942 |
| 21 | No change | 1.781 | 1.891 | 1.781 | 2.930 | 4.096 |
| 28 | Turbidity at $22^{0}$ and $37^{o}$C | 1.781 | 1.993 | 1.978 | 3.564 | 4.096 |
| No. of immunogenic components after 28 days | | 7 | 7 | 7 | 6 | 6 |

**Table 2:-** Effect of temperature on toxicity and immunogenicity of Indian common krait venom

| Property | | Venom Exposed to | | | | |
|---|---|---|---|---|---|---|
| | | $0^{0}$ | $4^{0}$ | $8^{0}$ | $22^{0}$ | $37^{0}$ |
| Toxicity % | R | 100 | 89.36 | 89.36 | 49.97 | 43.48 |
| | L | 0 | 10.64 | 10.64 | 50.03 | 56.52 |
| Immunogenicity % | R | 100 | 100 | 100 | 85.71 | 85.71 |
| | L | 0 | 0 | 0 | 14.29 | 14.29 |

Abbreviations: R – Retained, L - Lost

The venom turns turbid when stored at a temperature of 22°C and 37°C. Toxicity was retained up to 49.97% at 22°C and upto 43.48% at 37°C. The temperature $4^{0}$Cand $8^{0}$ C, showed similar results and 89.36% toxicity was retained in both cases. Immunological components were retained at $0^{o}$C, $4^{o}$C and $8^{o}$C. No antigenic component was lost at $0^{o}$C, $4^{o}$C and $8^{o}$C after storing the venom for 28 days.

## Discussion:-

From Table 2, it can be observed that the high temperature i.e. 22°C and 37°C have destroyed 50% and 56.52% of toxicity respectivelyand 14.29% of the immunogenicity is lostat boththetemperatures.Thevenom lost it's immunogenicity at 22°C and 37°C up to 14.29% only. The toxicity also declined at these temperatures.

As the venom is mainly composed of enzymatic proteins and are necessary for the venom to be toxic thereforewhen an enzyme is acted upon by the temperature variation, this leads to transformation of the venom and enzyme in particular. In normal conditions enzymes are needed for the proteins to be toxic. The action is activated when we increase the temperature of the reaction. But when venom is subjected to temperature variation for a long period of time, like in this case for 28 days, the effect is rather different and the toxicity as well as the immunogencitiy of the venom is altered. There may be structural changes in the enzymes which leads to reduced toxicity and loss of immunogenic components. The lower temperature favours the retention of toxicity and immunogenicity of the venom.

## Conclusion:-

For raising antrisera, storing of venom at $4^{o}$C and $8^{o}$C may be a good idea as there is retention of immunological components but reduction in toxicity of venom. The lower temperature could be used for prolonged storage when the venom is in solution form.

This knowledge can be used to alter the toxicity of venom before inoculating itin horses and other animals that are being utilised to raise antisera. This can prevent the local reaction at the site of inoculation in the animal.There is potential for further research in this area.

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