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Review Article

# SNAKE VENOM, ANTI-SNAKE VENOM & POTENTIAL OF SNAKE VENOM

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**ABSTRACT**

Many active secretions produced by animals have been employed in the development of new drugs to treat diseases such as hypertension and cancer. Snake venom toxins contributed significantly to the treatment of many medical conditions. Venomous snakes have a bad reputation and rightly so because of their often deadly bites. But what makes a snake's bite so deadly is the venom. Of the 3000 snake species, just over 600 are venomous. Snake venoms are made up of hundreds of different types of peptides, enzymes, and toxins. Each individual snake produces its own specific venom. There are two main types of venom viz. hemotoxins and neurotoxins. Hemotoxins target the circulatory system. They prevent clotting compounds from functioning correctly, which causes uncontrollable bleeding. Neurotoxins target the central nervous system. They stop muscles from working, which leads to suffocation. Venoms that are composed of neurotoxins are particularly deadly, as the proteins within them are able to disrupt the channels that allow ions to flow across neuron membranes. When these communication channels are disrupted, entire body systems can crash, leading to immediate death. Medicines derived from hemotoxins are used to treat heart attacks and blood disorders. These drugs also lead to decreased incidence of stroke, kidney disease, heart failure, and diabetes. Medicines derived from neurotoxins are used to treat brain injuries, strokes, and diseases such as Alzheimer's and Parkinson's. Understanding the connection between the snake venom proteins and particular receptors could have a profound impact on the development of new treatments for diseases such as Parkinson's, Alzheimer's, and various pain disorders. An attempt has been made to review snake venom, which are anti-snake venom plants & potential of snake venoms.

**Keywords:** Snake venom, Anti-venom, Milking process.

## INTRODUCTION

Snake bite is a public health hazard in India. In India on an average 250000 snake bites are recorded in single year. Based on their morphological characteristics including arrangement of scales, dentition, osteology, mycology, sensory organs etc., snakes are characterized into families. The snakes found in India show great biodiversity and their length varies from 6 mm to 10 mm, while weight ranges between few grams to several kilograms. Snakes occupied deserts, forests, marshy, swampy places, lakes, streams and rivers of difficult terrains. The families of venomous snakes are Atract aspididae, Elapidae, Hydrophidae and Viperidae. The major families in the India subcontinent are Elapidae which includes common cobra, king cobra and krait, Viperidae which includes Russell's viper, pit viper and saw-scaled viper and Hydrophidae (sea snakes) of the 52 poisonous species in India, majority of bites and consequent morbidity is attributable to 5 species viz. *Ophiophagus Hannah* (king cobra), *Najanaja*(common cobra), *Daboiarusellii*(Russell's viper), *Bungaruscaeruleus*(krait) and Echiscarinatae(saw-scaled viper)[1-3].

### Snake Venom

Snake venoms are secretion of venomous snake which are synthesized and which are stored in venomous gland. The glands which secrete the zootoxin is a modification of the parotid salivary gland and are situated on each side of head below and behind the eye encapsulated in muscular sheath. The glands have large alveoli in which venom is stored before being conveyed by the duct to the tubular fangs, through which it is injected. Snake venom is a combination of many different proteins, peptides and enzymes and they are generally not dangerous when ingested. Snake venoms are complex mixture of enzymatic and toxic proteins, which include phospholipase A2 (PLA2s), myotoxins, hemorrhagic metalloproteinases and other proteolytic enzymes, coagulant components, cardiotoxins, cytotoxins and neurotoxins [4-6].

### Composition of snake venom

Snake venom consists of protein, enzymes, neurotoxins, coagulants, anti-coagulants and substances with cytotoxic effects. It has acidic pH. Specific gravity is 1.03 and is water soluble. Phosphodiesterase A2 causes hemolysis by lysing cell membrane of RBCs. Oxidases and proteases are used for digestion. Snake venom contains inorganic cations such as sodium, potassium, magnesium and small amount of zinc, nickel, cobalt, iron. Zinc is necessary for anticholinesterase activity. Calcium is required for activation of enzyme like phospholipase. Two major classification of toxins found in snake venom include neurotoxins (those which affect nervous system) and Cyto-toxins (those that attack cells).

### Type of Snake Venom

Different species have different type's venom which depends upon its species, geographical location, its habitat, climate, age etc. There are three types of venom according to its effect viz. Haemotoxic, Cytotoxic & Neurotoxic.

- Haemo-toxic venoms are one which affects cardiovascular system
- Cytotoxic venoms targets specific cellular sites
- Neuro-toxic venoms harm nervous system of human body.

Enzymes present in snake venom hydrolyze protein and membrane components which lead to tissue necrosis and blood clotting [7].

### Anti-venom

The only available treatment against snake bite is the usage of anti-venom. The first anti-venom was developed by Alberte Calmette against the Indian cobra (NajaNaja). Anti-venom is made by immunizing mammals such as horse, goat, rabbit with particular snake venom and the specific immunoglobins are isolated from the blood.

The subject animal will undergo an immune response to the venom, producing antibodies against the venom's active molecule which can then be harvested from the animal's blood and used to treat envenomation. Ant venom is classified into two types. Monovalent ant venom when they are effective against a given species venom. Polyvalent when they are effective against a range of species [8].

**Snake Milking Process**

- Snakes can be milked according to a regular schedule, depending on the species. The interval between milking varies among producers and ranges from every 2 or 3 weeks to every 3 months.
- For very dangerous species, the use of short-acting general anesthesia or moderate cooling (15°C) during milking can be considered (e.g. inhaled sevofluorane or sevoflurane, halothane or even carbon dioxide) as it reduces the risk of accidents both to the snake and to the snake-handler.
- For the collection of venom, the snake's head is grasped between index finger and thumb, just behind the angle of the jaw, while the snake's body is held between the trunk and the arm of the snake handler.
- By applying gentle pressure, the snake's jaws are forced open, the fangs exposed. The fangs are pushed through a plastic/parafilm membrane hooked over the lip of a glass vessel, and venom is squeezed out.
- Any venom sample contaminated with blood should be rejected. After venom extraction, the fangs are carefully withdrawn from the collection vessel, while preventing damage to the mouth and dentition and avoiding the snake's impaling itself with its own fangs. After each venom milking, all materials used for milking should be sterilized with a flame, and then cooled with a draught of air before the next snake is milked.
- During milking, the wearing of protective clothing and a mask as well as vinyl gloves is recommended to prevent any accidents or infections

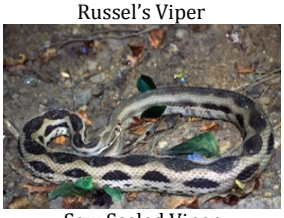
**Stability of anti-venom**

Liquid preparations have a shelf-life of up to 3 years at 2-8 °C, and freeze-dried preparations up to 5 years, when kept in the dark at room temperature. It is highly recommended that manufacturers perform stability studies to evaluate the possibility that their preparations could be stored for a long period under non-refrigeration (for instance at 30 °C). Real-time stability tests should be performed under the expected storage conditions of the anti-venom.

**Storage of anti-venom**

Anti-venom should be stored at a temperature within the range that assures stability, as found by stability tests. This is particularly critical for liquid formulations, which usually require storage at between 2 and 8 °C [9].

**Table 1: Type of snakes found and their features**

| Type of Snake found | Features |
|---|---|
| Common Krait  | • Found all across India uptoassam<br>• The venom of this snake is neurotoxic |
| Russel's Viper  | • Found all across Indian Subcontinent<br>• Its venom is haemotoxic |
| Saw Scaled Viper  | • Found across Indian Subcontinent except in West Bengal and Northeast India<br>• Its venom is haemotoxic |
| Spectacled Cobra  | • Found across Indian Subcontinent except Northeast India<br>• Its venom is neurotoxic |

**Therapeutic role of Anti-venom**

Many toxins from snake venom are investigated and formulated into drugs for the treatment ofconditions such as cancer, hypertension and thrombosis. Snake venom significantly lowers the blood pressure in human victims and experimental animals.

**1. Fibrinogenolytic and fibrinolytic activity**

Snake venom enzymes remove fibrinogen from the circulation without converting it to fibrin. Venoms with anticoagulant properties are extensively studied for possible medical applications. The drug Aggrastat (tirobifan) was developed from a compound in the venom of the saw-scaled viper (Echiscarinatus), and issued as an antiplatelet drug (glycoprotein IIb/IIIa inhibitors) [10].

**2. Cardiotonic and antiarrythmic activity**

Shermann et al observed that Malayan pit viper venom has blood thinning properties and could be effective in treating stroke patients.

Gomes et al identifies a non-protein micro molecular toxin from the Indian cobra. This toxin possesses antiarrhythmic properties at microgram level[11].

**3. Anti-Cancer activity**

Calmette et al investigated the use of cobra venom in the treatment of cancer in mice. In case of in vitro study, venom showed potent cytotoxic and apoptogenic effect on human leukemic cells (U937/K562) by reducing cell proliferation rate and produced morphological alterations [12-13].

**4. Muscle depolarization & Hemolysis activity**

Cytotoxin or Cardiotoxin are polypeptide of 60-70 amino acid residues long found in snakes of elapid family having various pharmacological effects such as depolarization of muscles, and hemolysis[14, 15].

**Side effects of anti-venom**

- Anaphylactic reactions such as difficulty in breathing, reddening of skin, swelling of eyes and face, fever
- Pyrogen reaction probably due to the action of high concentrations of non-immunoglobulin proteins
- Inflammation of joints, Enlargement of lymph gland [16].

**Plants used for Snake bite**

The plant constituents are used to neutralize the effects of snake venoms. The way of management of snake bites designed to control infection, stop pain, improve symptoms, correct imbalance, adjust immune system and boost energy for better health and quality of life.

**1. *Aristolochia odoratissima***

In the low lands south of Maracaibo Lake people drink an infusion of *Aristolochia odoratissima* leaves to treat snakebites.Toxicity studies showed that the aqueous extract of A. odoratissima did protect the mice against the lethal effects of Bothropsatrox venom. Nevertheless, protection was only observed at higher doses of venom (8 and 16 mg/kg), without modifying the values at the lower doses [17].

**Fig. 1: *Aristolochia odoratissima***

**2. *Tamarindus indicus***

Aqueous and alcoholic extracts of dried seed powder of *Tamarindus indicus* were tested for their antioxidant and inhibitory activity of toxic enzymes like PLA2 and proteinases of Najanaja venom. The methanolic extracts of *T. Indica*seed possess compounds, which inhibit the activity of Phospholipase A2 and Proteinases of cobra venom. It may be used as an alternative treatment to serum therapy and as a rich source of potential inhibitors of toxins involved in several pathological conditions of humans and animal diseases [18].

**3. *Holarrhena antidysenterica***

Jain and Srivastava have reported the use of the bark against snake bite. Prusti and Behera, in an ethno-medico-botanical study of Sundargarh District,Orissa, India, have reported the roots rubbed on a stone with a few drops of water and the paste obtained is given internally and applied externally in snakebite [19].

**Fig. 2: *Tamarindus indicus***

**Fig. 3: *Holarrhena antidysenterica***

**4. *Andrographis paniculata***

A. paniculata plant extract has anti-venom activity against *Najanaja* venom. The leaves of A. paniculata contains andrographolide, the active constituent of which is diterpene and is responsible for ASV property by modifying the actions of proteins, and enzymes also inhibit snake venom phospholipase $A_2$ activities [20].

**Fig. 4: *Andrographis paniculata***

**Tests to determine anti–venom activity**

The testing of plant extracts for anti-venom activity illustrates the traditional use of plants in treatment of snake bite. The variety of activity displayed by different snake venom systems requires different test systems to investigate inhibitory effects of plant extracts. A few studies were carried out where the extracts were given prior to injection of venom or after administration of venom, which is most analogous to the case of snake bite.

**Table 2: Plats having anti-snake venom activity**

| Sr. No. | Plant | Part used | Reference |
|---|---|---|---|
| 1 | *Piper longum* (Piperaceae) | Fruits | 21 |
| 2 | *Parkia biglandulosa* (Mimosaceae) | Stem bark | 22 |
| 3 | *Dichrostachys cinerea*(Mimosaceae) | Root | 23 |
| 4 | *Strychnos nux vomica* (Loganiaceae) | Seed | 24 |
| 5 | *Pouzolzia indica* (Utricaceae) | Aerial parts | 25 |
| 6 | *Bridellia ferruginea* (Euphorbiaceae) | Leaves | 26 |
| 7 | *Boswellia delzielli* (Burseraceae) | Stem bark | 27 |
| 8 | *Securidaca longipedunculata*(Polygalceae) | Root | 28 |
| 9 | *Sapindus saponaria* (Sapindaceae) | Callus | 29 |
| 10 | *Parinari curatellifolia* (Chrysobalanaceae) | Root bark | 30 |
| 11 | *Tamarindus indica* (Leguminosae) | Seed | 31 |
| 12 | *Mucuna pruriens(Fabaceae)* | Seed | 32 |
| 13 | *Curcuma longa* (Zingiberaceae) | Rhizome | 33 |
| 14 | *Pluchea indica* (Asteraceae) | Root | 34 |
| 15 | *Hemidesmus indicus*(Apocynaceae) | Root | 35 |
| 16 | *Guiera senegalensis* (Combretaceae) | Leaves | 36 |
| 17 | *Acalypha indica* (Euphorbiaceae) | Leaves | 37 |
| 18 | *Hibiscus aethiopicus*(Malvaceae) | Whole plant | 38 |
| 19 | *Magnifera indica*(Anacardiaceae) | Stem bark | 39 |
| 20 | *Symplocos cochinchinensis* (Simplocaceae) | Leaves | 40 |
| 21 | *Crinum jagus* (Amyrillidaceae) | Bulb | 41 |

**Various tests to determine anti venom activity**

**1. In vivo animal testing**

The protection of whole animals against a dose of venom by the plant extracts is impractical now-a-days because of ethical considerations. Recently mice have been used for the testing of crude extracts. A lethal dose of the venom was mixed with the varying doses of the plant extract and injected into the animal. Later the survival rate with and without extracts was determined.

**2. Testing using isolated organ preparations**

The test consists of measurements on nerve–muscle preparations, isolated muscles and studies on blood clotting procedures. The cobra venoms that impair neuromuscular transmission are experimentally studied using nerve muscle preparations from neck of chick (biventercervicis) and abdomen of the rat (phrenic nerve hemi diaphragm). Indirect stimulation of these preparations is inhibited by the venoms. Plant extracts containing anti-venom activity may consequently reverse these inhibitory effects. This was demonstrated with *Curcuma longa* extract against the neurotoxin from *Naja najasiamensis*. Envenomization by the Carpet viper, *Echiscarinatus* causes rapid intra - arterial clotting of blood, resulting in internal haemorrhage due to depletion of fibrinogen. *Mucuna pruriens* (Naikurana**;** Leguminosae) increased the clotting time of blood induced by *E. carinatus* venom.

**3. Tests using Enzymes**

The enzyme based assays were used for enzyme inhibition or enzyme activation of large numbers of plant extracts.The potassium salt of gymnemic acid isolated from *Gymnemasylvestre* (Asclepiadaceae) inhibits ATPase from cobra and viper venom. Inhibition occurs due to competitive binding between gymnemate and ATP [42, 43].

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