



MEDICINAL PLANTS ACTIVE AGAINST SNAKE ENVENOMATION

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ABSTRACT

Snakebite is an important cause of morbidity and mortality and is one of the major health problems in India. About 30000 to 40,000 persons die each year from venomous snake bite. Russell's viper or daboia (*Viper russelli*) appears to be the commonest cause of fatal snakebite in Southern India, Pakistan, Bangladesh, Sri Lanka, Burma and Thailand. Intravenous administration of anti-snake venom neutralizes the systemic actions, however, antiserum does not provide enough protection against venom induced hemorrhage, necrosis, nephrotoxicity and often develops hypersensitivity reactions. India has a rich tradition of the usage of medicinal plants. Many Indian medicinal plants are mentioned in Ayurvedic literature to treat snakebite victims and are used by many ayurvedic practitioners as well as in rural areas by traditioners. So much research work has been conducted for anti-snake venom activity of herbal medicine as alternative for Anti Snake Venom. This article presents a review of such herbal drugs which are effectively neutralize the snake venom like *vitex nigundo*, *Emblica officinalis*, *Hemidesmus indicus* etc which were assayed in research laboratories. It is considered as a valuable source of natural products for development of medicines against venomous snake bite.

Keywords: snake venom, plant extract, anti-snake venom, Venomous etc.

INTRODUCTION

The incidence of venomous snake bite is low in most developed countries but in India where the peoples are engaged in manual agriculture with exposed extremities the incidence of snake bite is higher. In India, data are fragmentary because less than 40% of snake-bite patients attend public hospital. *Echis carinatus* (the carpet or saw-scaled viper) occurs in both India and Pakistan, where it is responsible for a large number of snakebite cases, reaching 95% of envenomations in the State of Jammu. *V. russelli* also are frequently encountered in India and throughout south-east Asia. In Maharashtra State, in India, the annual incidence of severe envenomation is about 70 per 100000 inhabitants and the mortality rate is about 2.4 per 100000 per year. In Asia (population, ca. 3500 million) as a whole there may be up to 4 million snake-bites each year, of which almost 50% are envenomed. Approximately half of the victims reach hospital and the annual number of deaths resulting can be estimated at 100000¹. The upper bounds of recent annual estimates were 94,000 deaths globally and 15,000 deaths in India². The ASV (Anti-snake venom) treatment is being use in cases of snake bites. But antiserum does not provide enough protection against venom induced hemorrhage, necrosis, nephrotoxicity and often develops hypersensitivity reactions. There is currently no available monovalent Anti-snake venom primarily because there are no objective means identifying the snake species in absence of dead snake. To overcome the drawbacks of conventional antiserum, plant remedies against snakebite are being worked out as an alternative treatment. In Ayurvedic text a number of drugs are mentioned which shows anti- venomous effects. Experimental studies have proved that drugs like *vitex nigundo*, *Emblica officinalis*, *Hemidesmus indicus*, *Eclipta prostrate*, *Plucea indica* etc. are having venom neutralizing properties.

Classification of snakes

Snakes are broadly classified as poisonous and non-poisonous. There are five families of poisonous or venomous snakes, which are Colubridae, Elapiadae, Hydrophidae, Viperiade and Ataspidae. In India the four medically important poisonous land snakes are found i.e. Indian Krait (*Bengarus caeruleus*), common Cobra (*Naja-naja*), Saw scaled Viper (*Echis carinata*) and Russell's viper (*Viper russels*).³

In classical texts a detailed description of snakes, their classification, distinguishing factors are given. The snake are broadly classified into 5 varieties i.e. Darvikar, mandali, Rajimaan, Vaikaranj and Nirvisha⁴.

Anatomy

The typical snake- venom apparatus consist of paired venom glands one on each side of head below and behind the eye connected by ducts to hollow anterior maxillary teeth. In Viperiade, these teeth are large mobile fangs that retract against the roof of the mouth when animal is at rest. In Elapids and sea snakes the fangs are only slightly enlarged and fixed in an erect position. Vipers are characterized by some-what triangular heads, elliptical pupils, enlarged maxillary fangs. Immuno-diagnostic technique have been developed for species identification of snake involved in bites. An ELISA can be used to identify a specific type of snake –venom in victim's blood, wound aspirate or urine⁵.

Snake venom characteristics

Snake venom is very complex mixtures of enzymes, low molecule weight polypeptides, glycoprotein and metals ions. The enzyme and polypeptide affect the human body in multisystem fashion. Among these the deleterious components are haemorrhgins that render the vasculature leaky and thus cause both local and systemic bleeding⁵.

ANTI-SNAKE VENOM ACTIVITIES OF PLANTS

For the treatment of snake bite a number of herbal drugs and their combinations have been mentioned in

Ayurvedic texts. Some of the plants on which experimental studies were carried out are reviewed below.

Name of plant	Parts of Plant	Type of Extract	Type of snake venom	Reference
<i>Vitex negundo</i> and <i>Emblica officinalis</i>	Root	Methanol	<i>Vipera russellii</i> and <i>Naja kaouthia</i> venom	[6]
<i>Hemidesmus indicus</i>	root	Methanol	Viper venom	[7,8,9]
<i>Eclipta prostrata</i>		butanol	Malayan pit viper venom	[10]
<i>Pluchea indica</i>	root	Methanol	Viper and cobra venom	[11]
<i>Cordia verbenacea</i>		Methanol	<i>Bothrops jararacussu</i> snake venom	[12]
<i>Strychnos nux vomica</i>	seed	Ethanol	<i>D. russelii</i> and <i>N. kaouthia</i> venom	[13]
<i>Tamarindus indica</i>	seed	-	<i>V. russellii</i> venom	[14]
<i>Withania somnifera</i>	-	-	cobra (<i>Naja naja</i>) and viper (<i>Daboia russelii</i>) venoms	[15]
<i>Andrographis paniculata</i>	stem and leaf	Petroleum ether, ethyl acetate, methanol, and water.	<i>Naja naja</i> snake venom	[16]
<i>Morus alba</i>	Leaf	-	<i>Vipera/Daboia russelii</i>	[17]
<i>Mimosa pudica</i>	Root	Aqueous extract	<i>Naja naja</i> and <i>Bangarus caeruleus</i> venoms	[18, 19]
<i>Vitis vinifera</i> seeds	seed	Methanol	<i>Daboia/viper russelii</i>	[20]
<i>Mucuna pruriens</i>	seed	Distilled water	Cobra and Krait venoms	[21,22]

Vitex negundo* and *Emblica officinalis

The methanolic root extracts of *Vitex negundo* Linn. and *Emblica officinalis* Gaertn. were tested for antisnake venom activity. Both plant extracts were significantly neutralized the *Vipera russellii* and *Naja kaouthia* venom induced lethal activity both *in vitro* and *in vivo* studies. *V. russellii* venom-induced haemorrhage, coagulant, defibrinogenating and inflammatory activity was significantly neutralized by both plant extracts⁶.

Hemidesmus indicus

Hemidesmus indicus root extracts effectively neutralized Viper venom induced lethal, haemorrhagic, coagulant, anticoagulant and inflammatory activity.

An organic acid, isolated and purified from the root extract of an Indian medicinal plant sarsaparilla *Hemidesmus indicus* R. Br, possessed viper venom inhibitory activity. HI-RVIF significantly antagonized viper venom-induced lethal, haemorrhagic, coagulant and anticoagulant activity in experimental rodents⁷.

Lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* R.Br. could significantly neutralize lethality, haemorrhage, defibrinogenation, edema, PLA2 activity induced by *Daboia russellii* venom. It also neutralized *Naja kaouthia* venom induced lethality, cardiotoxicity, neurotoxicity and respiratory changes in experimental animals⁸.

2-Hydroxy-4-methoxy benzoic acid, isolated and purified from the Indian medicinal plant *H. indicus* possessed anti-snake venom activity. Rabbits immunized with *Vipera russellii* venom in the presence and absence of the compound along with Freund's complete adjuvant produced a precipitating band in immunogel diffusion and immunogel electrophoresis. The venom neutralizing capacity of this antiserum showed positive adjuvant effects as evident by the higher neutralization capacity (lethal and hemorrhage) when compared with the antiserum raised with venom alone⁹.

Eclipta prostrata

The butanolic extract, at 2.5 mg per mouse, was able to completely neutralize the lethal activity of 2LD₅₀ of MPV venom, but increasing the dose diminished the effect. The PBE, at 1.5–4.5 mg per mouse, was able to neutralize the lethality of the venom at around 50–58%. Both extracts

partially inhibited the hemorrhagic activity but displayed very low anti-phospholipase A₂ activity and did not inhibit proteolytic activity of MPV venom¹⁰.

Pluchea indica

The present study reports the neutralization of viper and cobra venom by beta-sitosterol and stigmasterol isolated from the root extract of *P. indica* Less. (Asteraceae). Anti-snake venom activity was studied in experimental animals. The active fraction was found to significantly neutralize viper venom-induced lethal, hemorrhagic, defibrinogenation, edema and PLA(2) activity. Cobra venom-induced lethality, cardiotoxicity, neurotoxicity, respiratory changes and PLA(2) activity were also antagonized by the active component. It potentiated commercial snake venom antiserum action against venom-induced lethality in male albino mice. The active fraction could antagonize venom-induced changes in lipid peroxidation and superoxide dismutase activity. This study suggests that beta-sitosterol and stigmasterol may play an important role, along with antiserum, in neutralizing snake venom-induced actions¹¹.

Cordia verbenacea

The methanolic extract from *Cordia verbenacea* significantly inhibited paw edema induced by *Bothrops jararacussu* snake venom and by its main basic phospholipase A₂ homologs, namely bothropstoxins I and II (BthTXs)¹².

Strychnos nux vomica

The whole seed extract of *S. nux vomica* (in low doses) effectively neutralized *Daboia russelii* venom induced lethal, haemorrhage, defibrinogenating, PLA2 enzyme activity and *Naja kaouthia* venom induced lethal, cardiotoxic, neurotoxic, PLA2 enzyme activity. The seed extract potentiated polyvalent snake venom antiserum action in experimental animals. Polyvalent snake venom antiserum action was significantly potentiated by the active compound. Spectral studies revealed it to be a small, straight chain compound containing methyl and amide radicals¹³.

Tamarindus indica

Tamarind seed extract inhibited the PLA2 (Phospholipase A₂), protease, hyaluronidase, l-amino acid oxidase and 5'-nucleotidase enzyme activities of venom in a dose-

dependent manner. The extract neutralized the degradation of the beta chain of human fibrinogen and indirect hemolysis caused by venom. Edema, hemorrhage and myotoxic effects including lethality, induced by venom were neutralized significantly when different doses of the extract were preincubated with venom before the assays¹⁴.

Withania somnifera

A hyaluronidase inhibitor glycoprotein (WSG) was purified from *W. somnifera*. The glycoprotein inhibited the hyaluronidase activity of cobra (*Naja naja*) and viper (*Daboia russelii*) venoms, which was demonstrated by zymogram assay and staining of the skin tissues for differential activity. WSG completely inhibited the activity of the enzymes at a concentration of 1:1 w/w of venom to WSG.

The glycoprotein inhibited the phospholipase A(2) activity of NN-XIa-PLA(2,) isolated from the cobra venom, completely at a mole-to-mole ratio of 1:2 (NN-XIa-PLA(2): WSG) but failed to neutralize the toxicity of the molecule. However, it reduced the toxicity as well as prolonged the death time of the experimental mice approximately 10 times when compared to venom alone. The interaction of the WSG with the PLA(2) is confirmed by fluorescence quenching and gel-permeation chromatography. Application of the plant extract on snakebite wound confirms the medicinal value associated with the plant.¹⁵

Andrographis paniculata

Shade dried stem and leaf parts of the *Andrographis paniculata* were extracted with different solvents on the basis of polarity nature such as petroleum ether (polarity 0), ethyl acetate (4.4), methanol (5.1), and water (9.0). All the four extracts were tested for their antivenom activities through *in vivo* experiments. Among those, methanol extract of *Andrographis paniculata* has shown significant inhibition on neurotoxic symptoms caused by the venom (450 µg/ kg b.w) and prolonged the survival time of mice (22 ± 2 g) maximum up to 14.44 ± 0.55 h compared to other extracts. This *in vivo* screened active methanol extract was further tested for direct inhibitory activity on *Naja naja* snake venom major enzymes like; acetyl cholinesterase, hyaluronidase, ATPase, protease, and hemolytic activities *in vitro*. In these experiments, the venom was preincubated with different concentrations of *Andrographis paniculata* methanol extract at 37 °C for 1h before adding to the reaction mixture *in vitro*. The results confirmed that, the methanol extract of *Andrographis paniculata* possesses potent snake venom inhibitors¹⁶.

Morus alba

In this investigation *Morus alba* plant leaf extract has been studied against the Indian *Vipera/Daboia russelii* venom induced local and systemic effects. The extract completely abolished the *in vitro* proteolytic and hyaluronolytic activities of the venom. Edema, hemorrhage and myonecrotic activities were also neutralized efficiently. In addition, the extract partially inhibited the pro-coagulant activity and completely abolished the degradation of A α chain of human fibrinogen. Thus, the extract processes potent antisnake venom property, especially against the local and systemic effects of *Daboia russelii* venom.¹⁷

Mimosa pudica

Study shows that *M. pudica* tannins are more effective than tannic acid in neutralizing 2 LD₅₀ of *N. kaouthia* venom *in vitro*, but less efficient than antivenom¹⁸.

Aqueous extract of dried roots of *Mimosa pudica* was tested for inhibitory activity on lethality, phospholipase activity, edema forming activity, fibrinolytic activity and hemorrhagic activity of *Naja naja* and *Bangarus caeruleus* venoms. The aqueous extract displayed a significant inhibitory effect on the lethality, phospholipase activity, edema forming activity, fibrinolytic activity and hemorrhagic activity. About 0.14 mg and 0.16 mg of *M. pudica* extracts were able to completely neutralize the lethal activity of 2LD₅₀ of *Naja naja* and *Bangarus caeruleus* venoms respectively¹⁹.

***Vitis vinifera* seeds**

In this investigation, the methanolic extract of grapes (*Vitis vinifera L.*) seed was studied against the Indian *Daboia/ viper russelli* venom-induced local effects. The extract abolished the proteolytic and hyaluronidase activities and also efficiently neutralized the hemorrhage, oedema-inducing and myonecrotic properties of the venom. In addition, the extract also inhibited partially the procoagulant activity of the venom and abolished the degradation of A α and B β chains of human fibrinogen. Thus the extract possesses potent anti-snake venom property, especially against the local effects of viper bites²⁰.

Mucuna pruriens

This research demonstrated very clearly that crude extract from *Mucuna pruriens* seeds contains principles which act as effective protectors, when they are injected intraperitoneally, at 24 hours or 14 days before the administration of a lethal dose of the venom²¹.

The antivenom potential of *Mucuna pruriens* plant extracts were checked against Cobra and Krait venom. Various pharmacological activities like lethality, edema forming activity, hemorrhagic activity, fibrinolytic activity, phospholipase activity (PLA2), procoagulant activity caused by Cobra and Krait venom were carried out. Neutralization of these pharmacological effects was carried out using *Mucuna pruriens* plant extract.

The results showed that the *Mucuna pruriens* plant extract was capable of neutralizing the lethality induced by the venom. The Cobra and Krait venom showed the presence of PLA2 enzymes by means of producing hemolytic haloes in indirect hemolytic assays. *Mucuna pruriens* plant extract was capable of inhibiting PLA2 dependent hemolysis of sheep RBCs in a dose dependent manner.

Edema-forming activity was assessed for Cobra and Krait venom and *Mucuna pruriens* seed extract was found to be effective in neutralization of edema induced by venoms. There was a significant decrease in the edema (footpad thickness) when there was an increase in the antivenom (plant extract) dose. Procoagulant activity induced by Cobra and Krait venom was studied using human citrated plasma and *Mucuna pruriens* seed extract was found to be effective in the neutralization of procoagulant activity. *Mucuna pruriens* seed extract was effectively antagonised the fibrinolytic activity. So *Mucuna pruriens* seed extract was effective in neutralizing the main toxic effects of the Cobra and Krait venoms²².

CONCLUSION

The most ancient system of medicine Ayurveda has described a number of drug for the cure of snake bite but no sufficient explanation of their mode of action was available. The recent experimental work not only prove of effectiveness of various herbal drugs on snake bite but also clearly show their mode of action along with active principal. These drugs are found to completely abolish both systemic and local effect of snake venom. No anaphylactic reaction observed in above studies which is very note worthy. Thus these drugs are better alternative for snake envenomation. Still a lots of work has to be done, there are still a number of drugs and combinations of drugs which are mentioned in Ayurvedic text to have anti-venomous properties. These drugs need to be identified and research work should be done on each plant and combinations (Yoga's) also so that alternative drug for Anti-snake venom will put forward.

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