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Review Article

Ophidian Bite: The Balance between Perception, Idealism and Realism

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ABSTRACT

Snakebite envenomation associated toxicity and death is an important and widely underestimated health issue in the African, Asian and some American provinces. It usually has great impact on larger population especially from remote, poorly developed tropical communities with the lowest quality of life indices. The only treatment available for this Neglected Tropical Disease (NTD) is the anti-snake venom (ASV) that has limitations for its usage due to its availability, affordability and associated adverse events. Hence, enhancement of current therapeutic regime and addition of multiple treatment options for snakebite management is inevitable to reduce the mortality and morbidity. The folk and traditional medicines especially plants, as herbal antidotes against snake bite have been in use for centuries with notable success. However, a comprehensive and ethnopharmacological approach is essential to study the various plants and their bioactive components needed to treat snakebite.

The review aims to provide compiled information about snake envenomation, biomolecules involved in mechanism of toxicity along with various plants and their constituents useful against snake bite. Related literature available on the various search engines as Google, Google Scholar, PubMed, Medline database, Science Direct, Research Gate were explored and thoroughly read for compilation of information. The review endeavored to provide in-depth knowledge about snake envenomation and its treatment strategies with focus on medicinal plants and its bioactive compounds useful to mitigate venom and future perceptions. It will surely help researchers in the field for development of better treatment options for snakebite envenomation and associated complications.

Keywords: Snakes, Antivenom, Traditional medicine, Snake envenomation.

Introduction

Snakebite is a major life-threatening disease of many tropical and subtropical provinces particularly; Sub-Saharan Africa, Southeast Asia, and Latin America. An estimated 5.4 million cases, 2.7 million poisonous cases, and 0.08 to 1.37 million death occurs annually because of snake bites.[1] The incidences of snakebites and associated deaths are high but the data available from many regions is often unrealistic and misleading because of lack of proper registration of snakebite.[2] The people engaged in farming, hunting, fishing and other rural activities are the most vulnerable population to the incidence of snakebite.[3,4] Because of issues relating to treatment costs, loss of earning capacity and ongoing disability, it is considered to be an occupational disease having high impact on economy in an economically vulnerable population.[5] Although, the epidemic potential of snakebite is low but the annual death rate due to snakebite is high than currently NTDs including dengue hemorrhagic fever, cholera, leishmaniasis, schistosomiasis, Japanese encephalitis, and Chagas disease.[6] The World Health Organization (WHO) had taken important step and included snakebite envenomation in its NTD list in June 2017.[1]

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The initiative had been taken to include right strategies for regional collaborative efforts to reduce the injuries due to snakebite incidence and mortality related to that. Community awareness and education is also included which is a more effective tool to save large population across the globe. The geographical location, seasonal differences, whether victim is healthy adult, teenager, or age-old individual, his/her food habit, body mass index are few factors which play important role in deciding the poisonous impact of the snake venom.[7] ASV therapy is best available cure against snake envenomation, but it is expensive, not readily available and has risk of immunological reactions.[8] These factors necessitate complementary therapies to treat snakebites. The treatment of snake envenomation using plants and folk medicine is old and ancient practice in many poor communities from rural areas.[9] Moreover, many evidences support the pharmacological use of plants, its extracts and isolated compounds against snakebites as medicine.[10-13] This review intended to provide more systematic collection of information on various medicinal plants and their components reported or used for treatment of snakebite. A thorough survey highlights the fact about medicinal uses of plants which may further help to identify novel ingredients having action on snake poison, thereby increasing the perception about its advances besides various other toxins. Besides, useful information is gathered about venomous snakes, snake venom, its effect, ASV and other treatments and their drawbacks to conceptualize the data.

Materials and Methods

The review was conducted based on the literature available on the internet that discussed about snake bite and its available treatments. The researchers used the search terms snakes, poisonous snakes, snakebite, snake taxonomy, snake envenomation, anti-venom therapy,

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pathophysiology of snake envenomation, pre-hospital management and first-aid measures for snake bite, traditional medicine, medicinal plants useful in snakebite etc. on Google, Google Scholar, PubMed, Medline database, ScienceDirect, ResearchGate search engines. The most relevant articles related to subject matters were included in review and otherwise were excluded.

Taxonomy of venomous snakes and their distribution

In biology, taxonomy plays an important role in defining living and existing organisms. For snakes it is useful in identification of more hazardous species, development of relevant immunotherapy and improvement of patient care. The classification of snakes based on taxonomy and poisonous nature is given in Figure1. The taxonomy is continuously evolving with multiple revisions over decades, as people are able to discover new species or some of the existing species are further categorized and sub-categorized according to unique characteristics. For example, *Tropidopheidae* family belonging to West Indies, Central and Southern part of America was consisting of four genera earlier, but now it contains, only *Tropidopheinae* having only two genera, *Tropidophis* and *Trachyboa*, containing 23 species. Similarly, *Acrochordus* a single genus from *Acrochordidae* family is composed of three species, *A. arafurae*, *A. granulatus*, and *A. javanicus* from Southern Asia and Australia, but is now sister taxon to the massive superfamily *Colubroidea.*[14] These changes indicate the diversity in snake types and population. This will further help to increase understanding of venom collectors, its producers, ASV manufacturers and investigators. More than 3000 snake species are available worldwide out of which around 250 are poisonous and more harmful.[15]

Historically, only the Indian cobra (*Naja naja*), Russell's viper (*Daboia russelii*), common krait (*Bungarus caeruleus*) and the saw-scaled viper (*Echis carinatus*) snakes were considered medically important in India. However studies showed the medical importance of other snakes such as hump nosed pit-viper (*Hypnale hypnale*), the Levantine viper (*Macrovipera lebetina*)[16-17] or *Trimeresurus (Peltopelor) macrolepis* from Tamil Nadu's hilly areas and Kerala, *Trimeresurus (Craspedocephalus) malabaricus* from southern India, *Naja kaouthia* in north east India,*Trimeresurus (T.) erythrurus* from Assam and Sikkim, *B. walli* and possibly *B. niger* found in north-west India, *Bungarus sindanus* commonly observed in west and north-west India etc.[18]

WHO has created database of snake species of higher medical importance which are widely distributed (Figure 2) and able to cause higher mortality and injury. This will enable users for easy identification of any venomous species from their country, territory and/or area.

Table 1 lists the venomous snake species with higher medical importance from each of four wide geographic regions. The toxicology department of Indian government is also following the WHO's model for defining snakes of medical significance. This will definitely help to increase the knowledge about type of snakes causing injuries and deaths in India. Hence, effective snakebite management strategies and remedies are required to be developed in near future.

Snake venomics and proteomics

Snake venom is combination of many proteins, lipids, carbohydrates, amines, peptides, enzymes and non-enzymatic components. The change in snake family, its geographical location, genus, species, age, size of snake and typical pre type, will result in changes in the composition of snake venoms.[20] The short front fangs snakes (Proteroglyphs) such as cobra, mamba, and coral snakes produces nerve toxins and causes neurotoxicity whereas long, hinged, hollow fangs (Solenoglyph) snakes like puff adder, Russell's viper, rattlesnakes etc. contains blood toxins and they are haemotoxic.[21] However, no snake possesses just a single type because each snake tends to have different combinations of toxins. In general, there are nearly twenty varieties of toxic enzymes found in snake venoms all over the world. Many of the snake venoms commonly contain six to twelve varieties of these toxic enzymes. Tasoulis and Isbister have extensively reviewed the snake venom proteomes from various available literature sources. They have identified 59 protein families and categorized them into different groups and subgroups based on their availability into particular snake species. Phospholipase A2 (PLA2), Snake Venom Metalloproteinase (SVMP), Snake Venom Serine Proteases (SVSP), and Three Finger Toxins (3FTx) were considered dominant protein families; Cysteine-rich secretory proteins (CRiSP), L-Amino acid oxidase (LAAO), Kunitz Peptides (KUN), C-type lectins (CTL), and natriuretic peptides (NP) were categorized as secondary protein families, nine minor protein families and 36 were considered rare protein families.[22] Table 2 shows the common proteolytic enzymes and other non-enzymatic constituents present is most of the snake venoms. Some of these enzymes are responsible for toxic effects in human being such as[21-32]

- ✓ Phospholipase (PLA2): Myotoxicity, oedema formation, anticoagulant effects, hypotension, flaccid paralysis, respiratory failure.
- ✓ Cholinesterase: Termination of neurotransmission by acetylcholine and attack on nervous system.
- ✓ L-amino acid oxidase (LAAO): Effects on platelet aggregation, inducing cell apoptosis, and antimicrobial activities.
- ✓ Proteases: Catalyse reactions that disrupt protein peptide bonds in tissues, causing blood-vessel wall damage and haemorrhaging and muscle fibre deterioration.
- ✓ Metalloproteases: Venom metalloproteases have physiological effects like tissue invasion, haemorrhage, necrosis (death of cell), and perhaps apoptosis.
- ✓ Three-finger toxins (3FTx): Postsynaptic neurotoxicity
- ✓ Polypeptide toxins: Directly disrupt nerve-impulse transmission, usually causing heart or respiratory failure.
- ✓ Hyaluronidases: Spreading factor" alters the structural, rheological, and chemical properties of the extracellular matrix.

The snake venom is also a rich source of pharmacological proteins having clinical efficacy. This potential has not been fully explored. However, the newer technologies such as venomics, proteomics, transcriptomics, metabolomics and genomics are progressing rapidly for characterization of overall snake venom composition.[33] This should provide further diversification and understanding of snake venom compositions and its biological active proteins for the treatment of heart disease, inflammation, cancer therapy or as a new tool for clinical diagnostic parameters.

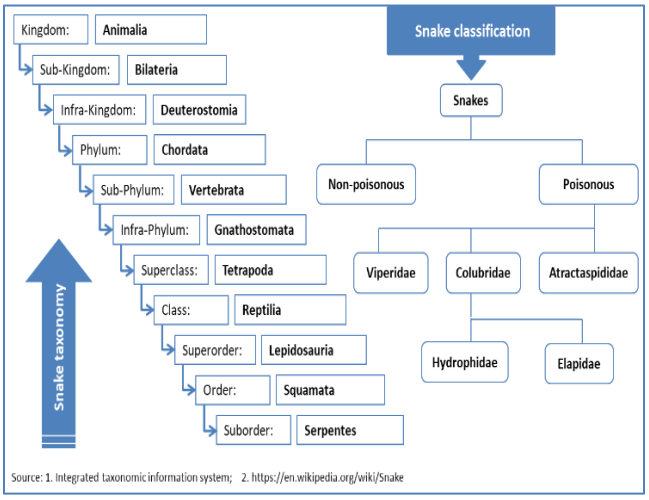

Figure 1: Taxonomic Hierachy of snakes

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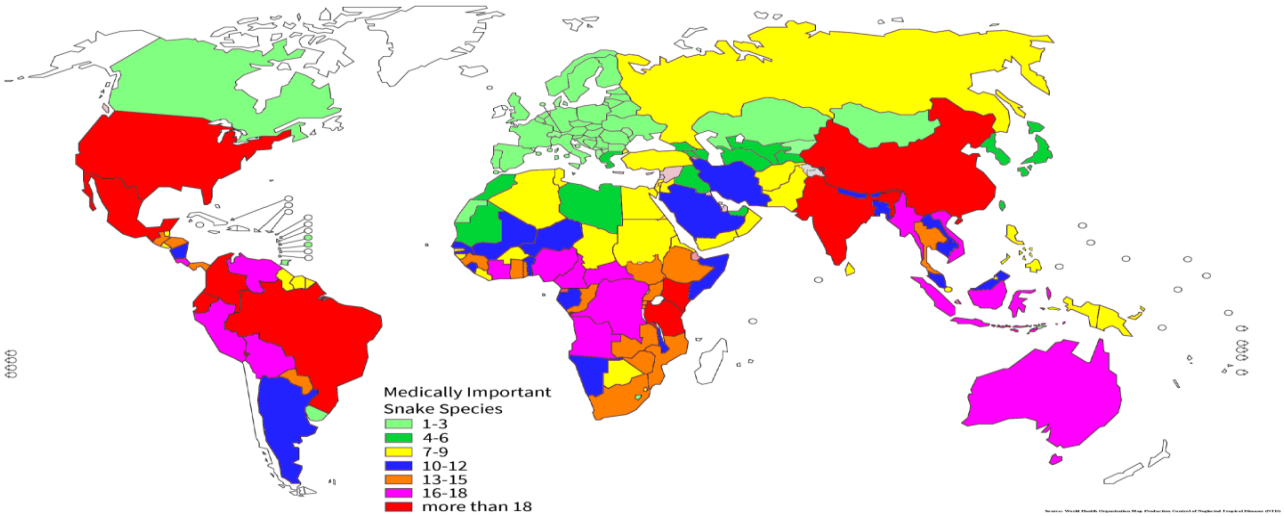

Figure 2: Global distribution of poisonous snakes

Pathophysiology and management of snake envenomation

The toxicity of snake venom cannot be attributed to any single component. Some venom components act as antagonist while other shows synergistic effect. Binding of these components to multiple enzymes, receptors or molecular targets creates disturbances which may affect several body systems through complex mechanism. Neurotoxicity, Heamatoxicity and Myotoxicity are key features of snake envenomation but some of their manifestations are still not elucidated. Many components of snake venoms can result in cellular penetration leading to cell inflammation, release of various chemical mediators and occurrence of other unusual responses which are initiated by entry of venom through vessel wall.[34] Spontaneous systemic bleeding, coagulopathies, affected platelets and damage vascular tissue due to snakebite may lead to major blood loss and death. It also induces prominent local tissue damage including severe bleeding, coagulation, cardiac shock and kidney failure.[35] Many snakebite patients can develop ptosis and extraocular neuromuscular weakness due to non-depolarising pre-synaptic and post-synaptic blockade.[36] Figure 3 shows the overall pathophysiology of snakebite.

Although, the ASV could be first choice for treatment of systemic snakebite envenomation, other treatment modalities such as first-aid measures like tourniquets, venom extractors, electric shock, pressure immobilization bandage, incision of bite wound, black stone and herbal medicines could also be fundamental to achieve better outcomes. It is important to give reassurance to bitten victim considering the usual delays in reaching the patient to nearest hospital. Determination of snake type and bite time will be helpful in understanding the toxic effect. Collection of brief history about bite and examination of progression of local pain and systemic symptoms like bleeding or blister formation at bite site is required. In hospital patient will be observed for breathing, circulation and possibility of shock. However, in country like India, doctors working in primary health care centers are facing difficulty in treating the snakebite patients due to lack of confidence and lack of treatment protocols. In the secondary and tertiary care hospitals, multiple treatment protocols are being followed, predominantly based on western textbooks.[37] In view of the same, Government of India has developed a National Snakebite Management Protocol which will guide and help doctors and other individuals for providing best possible treatment to the snakebite victim.[38]

Antivenom therapy

Intravenous use of antivenom is the most acceptable treatment against snakebite. It was developed almost 125 years ago though it is one of the most unusual pharmaceutical agents for treatment in human patients. Various animal species like horse, donkey, sheep, rabbit, goat etc. are used for production of antivenoms. However, horse is the preferred choice for large scale and commercial production of antivenom because they are able to produce large volume of plasma, less susceptible to disease, more adaptable to local environment, easily available, and also cost of their maintenance is less. Most of the conventionally antivenoms are obtained by administering the attenuated or non-lethargic dose of venom to animal species. The blood sample from hyperimmunized animals is collected. The separated serum from this blood contains antibodies.[39-40] In earlier years, the purification procedure was not well developed for preparation of antivenoms using animal serum. Later, it was improved a lot, so as to reduce the chances of immediate adverse reactions and serum sickness for its commercial development.[41] This purified serum contains either IgG immunoglobulin or antibody fragments such as Fab2 or Fab IgG which are effective in neutralizing the venom proteins. In addition, scientist has also developed and evaluated avian antivenom consisting of IgY immunoglobulin. The cost of production of this antivenom is less and more ethical because of use of chicken eggs instead of bigger animals for this development.[42-43] Camelid IgG and non-IgG components are found to be less reactogenic and more stable at high temperature. Thus, they are considered as potential alternative antivenoms.[44-45] Figure 4 identify the difference between conventional way of ASV development and advancement in various steps for production of modern or next generation antivenoms.

The effectiveness of any antivenom depends on its capacity to either stop the development of any clinical symptom or reversal of effect of snake venom. However, it is associated with immediate hypersensitivity reactions like urticaria, rash, tachycardia, hypotension, bronchospasm or angioedema. It can also cause delayed reactions of fever, vasodilatation, vomiting, myalgia, swelling, diarrhoea, itching etc. Due to large intra and inters species differences in snake and composition of their venom; the manufacturing of ideal therapeutic ASV is difficult. The therapeutic potential of two polyvalent antivenoms should not be considered identical even they have been prepared using same source of venom. For example, in recent study the variation was observed in neutralization capacity of two Indian polyvalent antivenoms against the Indian and south-east Asian *Naja* and *Bungarus* venoms. These antivenoms were manufactured by Vins Bioproducts and Bharat Serums and Vaccines.[46] Similarly, in Rajasthan large doses of ASV are requires for big northern sub-species of the saw-scaled viper (*Echis carinatus sochureki*) compared to smaller southern sub-species (*E. c. carinatus*).[17] Also, in Kozhikode, 10-20 vials (generally accepted initial doses) of polyvalent ASV failed to prevent development of capillary leak syndrome.[47] The monospecific (effective against single or closely related snake species) could be the solution for this. Considering the geographic diversity of medically important snakes, polyspecific (effective against multiple snake species) antivenoms may provide answer to some extent.

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Table 1: List of venomous snake species with highest medical importance [19]

Geographical region		Family	Species
Africa & Middle East	North Africa/Middle East	*Atractaspididae*	*Atractaspis andersonii*
		Elapidae	*Naja arabica, Naja haje, Naja oxiana*
		Viperidae	*Bitis arietans, Cerastes cerastes, Cerastes gasperettii, Daboia mauritanica, Daboia Palaestinae, Echis borkini, Echis carinatus, Echis coloratus, Echis omanensis, Echis pyramidum; Macrovipera lebetina, Montivipera xanthina, Pseudocerastes persicus*
	Central sub-Saharan Africa	*Elapidae*	*Dendroaspis jamesoni, Dendroaspis polylepis, Naja anchietae, Naja haje, Naja melanoleuca, Naja nigricollis*
		Viperidae	*Bitis arietans, Bitis gabonica, Bitis nasicornis, Echis leucogaster, Echis ocellatus, Echis pyramidum*
	Eastern sub-Saharan Africa	*Elapidae*	*Dendroaspis angusticeps, Dendroaspis jamesoni, Dendroaspis polylepis, Naja anchietae, Naja annulifera, Naja ashei, Naja haje, Naja melanoleuca, Naja mossambica, Naja nigricollis*
		Viperidae	*Bitis arietans, Bitis gabonica, Bitis nasicornis, Echis pyramidum*
	Southern sub-Saharan Africa	*Elapidae*	*Dendroaspis angusticeps, Dendroaspis polylepis, Naja anchietae, Naja annulifera, Naja mossambica, Naja nigricincta, Naja nivea*
		Viperidae	*Bitis arietans*
	Western sub-Saharan Africa	*Elapidae*	*Dendroaspis jamesoni, Dendroaspis polylepis, Dendroaspis viridis, Naja haje, Naja katiensis, Naja melanoleuca, Naja nigricollis, Naja senegalensis*
		Viperidae	*Bitis arietans, Bitis gabonica, Bitis nasicornis, Bitis rhinoceros, Cerastes cerastes, Echis jogeri, Echis leucogaster, Echis ocellatus*
Asia and Australasia	Central Asia	*Elapidae*	*Naja oxiana*
		Viperidae	*Echis carinatus, Gloydius halys, Macrovipera lebetina*
	East Asia	*Elapidae*	*Bungarus multicinctus, Naja atra*
		Viperidae	*Cryptelytrops albolabris, Daboia siamensis, Deinagkistrodon acutus, Gloydius blomhoffii, Gloydius brevicaudus, Protobothrops flavoviridis, Protobothrops mucrosquamatus, Viridovipera stejnegeri*
	South Asia	*Elapidae*	*Bungarus caeruleus, Bungarus niger, Bungarus sindanus, Bungarus walli, Naja kaouthia, Naja naja, Naja oxiana*
		Viperidae	*Cryptelytrops erythrurus, Daboia russelii, Echis Carinatus, Hypnale hypnale, Macrovipera lebetina*
	South-East Asia (excluding Indonesian West Papua)	*Elapidae*	*Bungarus candidus, Bungarus magnimaculatus, Bungarus multicinctus, Naja atra, Naja kaouthia, Naja mandalayensis, Naja philippinensis, Naja samarensis, Naja siamensis, Naja sputatrix, Naja sumatrana*
		Viperidae	*Calloselasma rhodostoma, Cryptelytrops albolabris, Cryptelytrops erythrurus, Cryptelytrops insularis, Daboia siamensis, Deinagkistrodon acutus*
	Australo-Papua (includes Indonesian West Papua)	*Elapidae*	*Acanthophis laevis, Notechis scutatus, Oxyuranus scutellatus, Pseudechis australis, Pseudonaja affinis, Pseudonaja mengdeni, Pseudonaja nuchalis, Pseudonaja textilis*
Europe	Central Europe Eastern Europe Western Europe	*Viperidae*	*Vipera ammodytes* *Vipera berus* *Vipera aspis, Vipera berus*
The Americas	North America	*Viperidae*	*Agkistrodon bilineatus, Agkistrodon contortrix, Agkistrodon piscivorus, Agkistrodon Taylori, Bothrops asper, Crotalus adamanteus, Crotalus atrox, Crotalus horridus, Crotalus oreganus, Crotalus simus, Crotalus scutulatus,*

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	Crotalus totonacus, Crotalus viridis
Caribbean	*Bothrops cf. atrox (Trinidad), Bothrops caribbaeus (St Lucia), Bothrops lanceolatus (Martinique), Crotalus durissus (Aruba)*
Central America	*Bothrops asper, Crotalus simus*
South America	*Bothrops alternatus, Bothrops asper, Bothrops atrox, Bothrops bilineatus, Bothrops brazili, Bothrops diporus, Bothrops jararaca, Bothrops jararacussu, Bothrops leucurus, Bothrops mattogrossensis, Bothrops moojeni, Bothrops pictus, Bothrops venezuelensis, Crotalus durissus, Lachesis muta*

Table 2: List of snake venom components [21-32]

Most Common components	Other Components	
• Phospholipase A_2	• Glutaminyl cyclase	• Flavine monoamine oxidase
• Serine proteases	• Aminopeptidase	• Lysosomal acid lipase A
• Snake venom metalloproteases	• Endonuclease	• Fibrinogenases
• Three-finger toxins (3FTx)	• Transferrin	• Haemoglobins
• L-Amino acid oxidase	• Waprin	• Neurotrophin
• Phospholipase B	• Endopeptidase	• Aspartic protease
• Natriuretic peptides (NP)	• Glutothione peroxidase	• Cysteine-rich secretory proteins (e.g. tripurin)
• Phospholipase C	• Kazal-type inhibitor	• C-type lectins/snaclecs
• Phosphomonoesterase	• Galactose-binding protein	• Acidic phospholipase A2 5,6
• Arginine ester hydrolase	• Trypsinogen	• Disintegrin trigramin-gamma
• Acetylcholinesterase	• Albumin	• Pallase
• Collagenase	• Prokineticin	• Snaclec coagulation factor IX-binding protein
• Hyaluronidase	• Selectin	• Chemokine receptor binding
• 5'-Nucleotidase	• Peroxiredoxin	• RNase
• Lactate dehydrogenase	• Protein c activator	• DNase
• Adenosine triphosphatase	• Polyglycine peptides	
• Kunitz peptides (KUN)	• Glycine-histidine rich peptide	

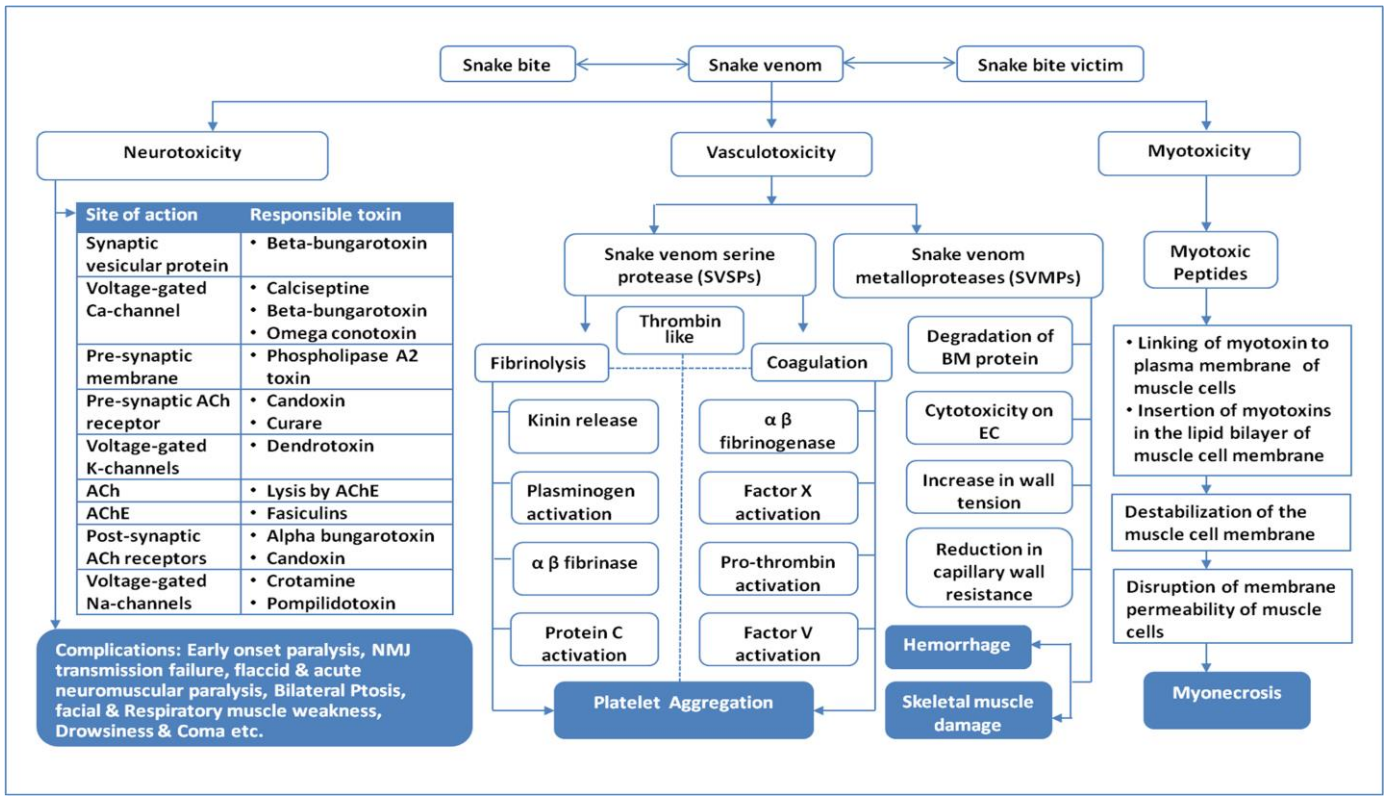

Figure 3: Pathophysiology of snake envenomation

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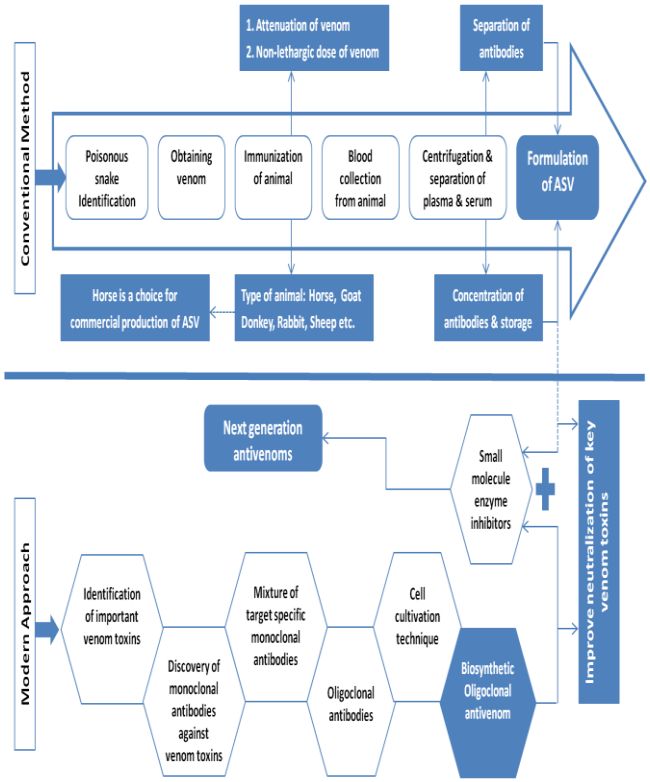

Figure 4: Approches for use of ASV in treatment of snake bite

From the commercial angel, the cost of development of antivenom is high and it is time consuming process. Liquid form of ASV is less stable requiring storage temperature of 0-4^0 C and can deteriorate early, leading to unfit for use as compared to its lyophilized form. Nowadays, multiple countries are producing the antivenoms and claiming that it is able to neutralize venoms form various animal species. However, there are very few organizations which are producing the ASV across the world. Moreover, because of the lower profit margin and unavailability of fix or stable market in terms of manufacturing and supply especially to the developing countries, many players have stopped or reduce the ASV production. This could be one of the reasons for availability of ASV.

The venom neutralizing capacity of antivenom against medically important snake should be carefully evaluated in animal pharmacology and toxicology studies before clinical studies in human. However, the preclinical studies do not represent the real-life scenario of snakebite. Similarly, due to differences in pathological and pathophysiological expressions because of multiple venom toxins the physiological variation and treatment strategies may differ in humans and animal species following envenomation. There are relatively very few animal studies which have been conducted for determination of LD_{50} and ED_{50}. These studies found to be important and more relevant in analyzing the preclinical efficacy of antivenom.[48] On the other hand, testing of antivenom in healthy volunteers in Phase-I study could be dangerous. More valuable and accurate data comes from Phase-II and III studies which include the use of comparator, primary objectives, inclusion/exclusion criteria, clinical endpoints, ethical consideration, statistical power, blinding to avoid bias, and statistical methodology for analysis. The literature search from Medline yielded very few trials which are being conducted in last ten years. Most of the studies lack controlled arm, defined primary outcomes, snake authentication or blinding. It also includes very small number of patients. Many ASV trials were conducted with or without placebo or using low or high dose of ASV as a comparator with major ethical issues.[49-61] It thus shows that, despite the number of published randomized, comparative and other observations studies, the efficacy and safety of some of the antivenoms is still questionable due to lack of robust clinical data. Majority of currently available ASVs were registered and marketed without first being studies clinically.[62] Well-designed clinical trials in human subjects are required to be conducted to produce quality data having enough evidence for effectiveness of antivenoms.

Next generation biosynthetic oligoclonal antivenom (BOA) which contains the mixture of human monoclonal antibodies is the improved therapy for treatment of snakebite. It has distinct advantages such as, it is compatible with human victims as it contains only human antibodies, it can be selected precisely for toxin neutralizing ability, safety profile is good, consistent reproduction is possible, rapid administration of antivenoms, continuous supply of snake venoms can be restricted and more acceptance among clinicians etc.[63] A problem with monoclonal antibodies is that they are not able to neutralize different venom toxins and are effective against single targets (monoclonal). This is a major difference between monoclonal and polyclonal and also the reason for efficacy of polyclonal antibodies.[64] Similarly, many synthetic and natural small molecules have been evaluated for its effect against various snake venom enzymes like L-amino acid oxidases, nucleotidases, and hyaluronidases either in combination with conventional antivenom or BOA having faster tissue penetration. Like BOA it is able to target only specific group of toxins.[42] Antibody fragments has also proven its efficacy against snake venom, however because of shorter half-life its repeat dose may be required for human use.[65]

Pre-hospital management of snake bite

Timely treatment of snake envenomation is the key for survival of snakebite victim. However, the transportation of victims to nearby medical facility, availability and stocking of antivenom, trained medical staff for antivenom administration and poorly developed emergency medical services are major hurdles. Snake venom could be injected in an artery, vein, muscle, subcutaneous tissue and result in rapid onset of toxicity. It has been observed that, the lymphatic channels are major route for spread of the venom from extremity into the systemic circulation.[66] All these factors have led to requirement of supportive treatment such as tourniquets, venom extractors, oral suction, electric shock, pressure immobilization bandage, incision of bite wound, black stone, cryotherapy etc. which will increase the possibility of delaying the onset of toxicity. Despite much of scientific rationale for their use, these treatment therapies are popular in many cultures and used by various communities. These techniques are also supported and evident in many past medical reports and works and hence often applied extensively but incorrectly. Unfortunately, many of these techniques are associated with unobserved risk, some of which can be harmful to the patients and are not effective against snake bite. As per WHO guidance for snake bite management,[9] many first-aid treatments measures used for snake envenomation across the world are found to be less beneficial and more harmful. Their use should be stopped, and it should not be permitted to delay the onset of toxicity while taking snake bite victim to nearby healthcare center or hospital. However, providing reassurance to patients and immobilization of bitten limb or whole body and providing immediate medical support to victim are some of the recommended first-aid strategies. Moreover, WHO has also contraindicated the arterial tourniquets, American Heart Association does not recommend the usage of mechanical suction for pit viper bite. The United States Food and Drug Administration (USFDA) has stopped snake bite treatment using electric shock.[67] On the other hand, topical nitric oxide inhibitor, glyceryl trinitrate ointment (GTNO) and injection of trypsin as a pharmacological treatment shown delayed toxicity in experimental design but requires further evaluation. A pressure immobilized bandage are likely to provide some benefits however, its usage is banned as first-aid treatment for North American Crotaline envenomation. This is as per American College of Medical Toxicology, American Academy of Clinical Toxicology, American Association of Poison Control Centers, European Association of Poison Centers and Clinical Toxicology, International Society of Toxinology, and the Asian Pacific Association of Medical Toxicology who have concluded that pressure immobilization is supposed to cause more serious adverse events after its use and it also lacks the efficacy in humans. Hence the pre-hospital management using pressure immobilization is not recommended.[68]

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Table 3: List of herbal bioactive compounds active against snake bite

Compound	Plant Source	Mode of action/Use	Reference
Aristolochic acid	*Aristolochia indica* *Aristolochia sprucei*	• Inhibits the lytic activity, • The edematose properties of some phospholipases of snake venoms	[98-100]
2-OH-4-methoxy benzoic acid	*Hemidesmus indicus*	• Anti-snake venom	[101]
Rosmarinic acid	*Cordia verbenacea*	• Inhibition of PLA2 activity	[102]
Beta sitosterol & Stigmasterol	*Pluchea indica*	• Anti-snake venom	[103]
Wedelolactone	*Eclipta prostrata* L.	• Anti-snake venom	[104-105]
Turmerin	*Curcuma longa* L.	• Inhibits the enzymatic activity, • Neutralizes cytotoxicity, oedema & myotoxicity	[106]
WSG	*Withania somnifera*	• Inhibition of PLA2 activity	[107]
Benzoylsalireposide and Salireposide	*Symplocos racemosa*	• Inhibition of Phosphodiesterase-I activity	[108]
4-nerolidylcatechol	- *Piper umbellatum* -*Piper peltatum*	• Inhibition of PLA2 activity	[109]
Ellagic acid	*Casearia sylvestris*	• Anti-snake venom against Bothrops genus	[110]
Gymnemic acid	*Gymnema Sylvestre*	• Inhibits ATPase venom	[111]
Lupeol acetate	*Hemidesmus indicus* R.Br.	• Inhibition of PLA2 activity	[112]
Alternamin	*Murraya alternans*	• Anti-hemorrhagic	[113]
Edunol	*Brongniartia podalyrioides*	• Anti-snake venom	[114]
Quinovic acid-3-*O*-alpha-L-rhamnopyranoside	*Bridelia ndellensis*	• Inhibition of Phosphodiesterase-I activity	[115]
Manoalide	*Luffariella variabilis*	• Inhibition of extracellular PLA2 activity	[116]
Ehretianone	*Ehretia Buxifolia*	• Anti-snake venom	[117]
Triterpenoid saponin	*Pentaclethra macroloba*	• Inhibition of antiproteolytic, • Inhibition of antihemorrhagic • Inhibition of metalloproteases activity	[118]
Ursolic acid	*Eriobotrya japonica*	• Inhibition of PLA2 activity	[119]
Solanidane	*Solanum campaniforme*	• Hemorrhagic inhibitor, • Necrotizing & myotoxicity effects	[120]
Dolastane	*Canistrocarpus cervicornis*	• Inhibition of induced hemorrhaging, Hemolysis & coagulation	[121]
Other important components having antivenom activity [122-123]			
Schumanniofoside	Glycyrrhizin	8-Methoxy coumestrol	Fucoidan
Resveratrol	Mimosine	Oleanoic acid	Quercetin
Chlorogenic acid	Vanilic acid	2,4 dimethyl hexane	Tannic acid
Ajmaline	Anisodamine	Quinic acid	Genistein
Catechin	Anisic acid	4-Nerolidyl-catechol	Melanin
Luteolin	Salireposide	Myricetin	Ehretianone
Apigenin	Gallic acid	Kaempferol	Lineorol
Tectoridin	Rutin	Serpentine	Hydrangenol

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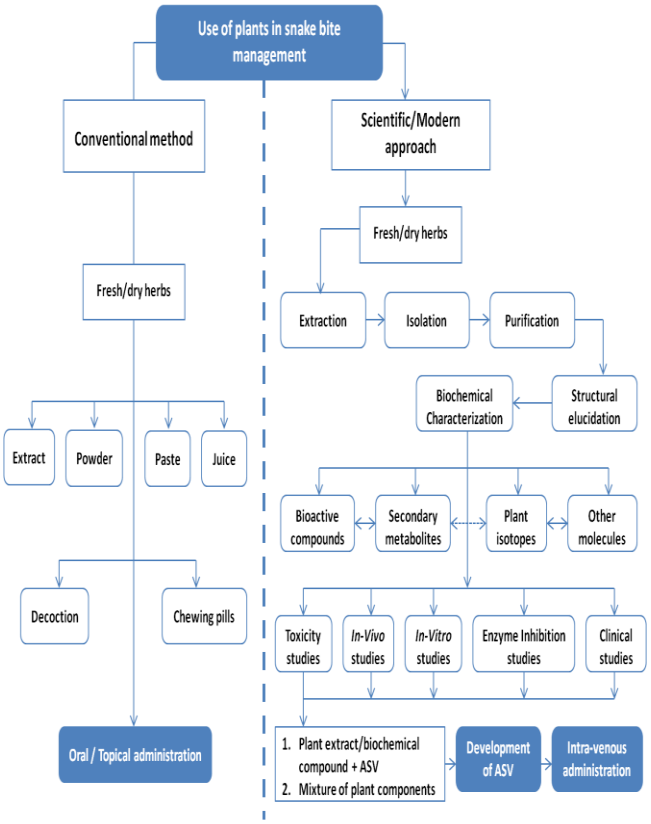

Figure 5: Approches for use of plants in treatment of snake bite

Beside this, pressure immobilization technique has not indicated any improvement against snake envenomation in any experimental study when assessed individually. Stopping the movement of bitten area using immobilization along with bandaging and supporting techniques (without compression) may benefit the patient. Nevertheless, no rigorous trials have validated this practice.[69] Plant extracts used in traditional medicines are shown to be useful in experimental studies and in real life situation and hence can be considered for use in rural areas.

Herbal treatment for snake bite

Use of plants as medicine is ancient practice and many Indians, Chinese, Babylonians, Hebrews, Egyptians and Assyrians communities are acquainted with it since 5000 B.C.[70] According to the WHO, more than 80% of the people in the world depends on traditional medicine for their initial healthcare needs.[71] In almost every part of the world wherever snakebite occurs, various plants species are used as herbal medicines to treat snakebite. The first scientific investigation of its kind was carried out by Knowles for use of herbal antidotes. He has observed the activities of various plant or their components used by local healers, but was not able to document its effectiveness in snake envenomation.[72] Further, Mhaskar and Caius tried to check the pharmacological activity of around 314 plants with their 184 combinations as herbal remedies for lethality although, the systemic changes caused after snake venom were not taken into account and lacks the supportive evidences.[73] Afterwards, many authors have found out the versatility of antivenom plants such as Rizzini *et al*. enlisted 83 species, Mors compiled 578 species, Martz *et al*. enlisted 11 species, Duke enlisted 470 species, Gomes and Dey and De enlisted several flowering plants that are active against snake venoms.[12, 74-77] Hashimoto in an ethnobotanical database enlisted 66 species of plants belonging to 31 families which are used in the Brazilian folk medicine as antidotes against snake venoms.[78] Large numbers of available Indian medicinal plants are effective against many infections and diseases. For example, many studies containing ayurvedic treatment strategies are ongoing in India against pandemic Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection which has caused in highest mortality and morbidity.[79-84] Also, other available literature highlights the treatment of snake envenomation using different plants by various local communities across India. For instances, the Malapandaram tribe of Kerala uses leaves of *Cipadessa baccifera* mixed with pepper and administer orally against snake bite.[85] *Rhapidophora pertusa* has analgesic and anti-inflammatory effects that help to reduce pain caused by snake bite.[86] The Sugali tribes of Andra pradesh, India use various species of ethnomedicinal plants in the form of paste, juices for snake bite.[87] Kunjam *et al*. described the application of herbal medicines by the Cherwa and Pando tribes of Chattisgarh, India.[88] The main tribal communities of Rajasthan, India viz., Bhil, Meena, Garasia, Damor, Sahariya and Kathodia use nearly 44 plants for the treatment of snake bite.[89] Herbal therapy of the tribal also involved oral consumption of juice leaf of *Calotropis gigantea (L.)*by Tripura tribes in Northeast, India.[90] In Maharashtra, India many plants are used by Kokni tribes as antidotes for snake bite and scorpion sting.[91] Some of the most cited plant families for use in snakebite are Fabaceae, Asteraceae, Apocynaceae, Lamiaceae, Rubiaceae, Euphorbiaceae, Araceae, Malvaceae, and Acanthaceae.[92] Moreover, in one of the human clinical studies of krait and viper bites; ayurvedic tablet formulation containing extract of Erythrina indica, Magnefera indica, Eugenia jambolana, and Jusminum sambac plant; known as Pinak shows that all cases on PINAK group recovered well and rapidly after snake envenomation in Maharashtra.[93]

The conventional approach for use of medicinal plants includes aqueous, methanolic or ethanolic extract, powder, paste, chewing pills etc. to reduce the effect of snake venom. Route of administration can be topical or oral. In contrast, the modern approach includes the separation of bioactive compounds from plants and its further evaluation. Figure 5 specify steps involve in conventional method and the scientific approach adapted for use of plants in snake bite and development of anti-venin drugs. Hence natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries. Studies shows that many secondary metabolites isolated from plants are useful in development of phytotherapetuics and designing the medicines.[94-97] Flavonoids, alkaloids, glucosides, organic acids, glycosides, tannins, volatile oils, resins, proteins, enzymes, trace elements, amino acids, phytochromes, polysaccharides are major chemical groups found in most of the plants which are active against snake envenomation.

These compounds neutralized the snake venom either by protein precipitation, enzyme inhibition, chelating action, adjuvant activity, antioxidant, anti-inflammatory, anti-hemorrhagic or antimyotoxic activity. Table 3 summarizes list of selected bioactive compounds effective against snake venom.

In general, use of traditional medicine in snakebite is purely based on either having availability of written documented proof or verbal information from historic practice. The development of antivenom is challenging process and use of medicinal plants for its preparation pose further difficulty in terms of time consumed, money spend and effectiveness of finished formulation. The estimated contribution of plant drugs in developed countries like Unites States is approximately 25% whereas in India and China the contribution is more than 80%.[124] In spite of their extensive use the snakebite and related complications are remains challenging to treat and no bioactive compound, secondary metabolites or other molecules is yet identified to neutralize snake venom completely. A drug discovery from plants can be fastened using newer and cutting-edge technologies. The bioactive compounds, secondary metabolite or deoxyribonucleic acid (DNA) molecular markers derived from plants are latest essential tools in the search of new medicines for snakebite. Many medicinal plants have been primarily studied for possible antiophidic effects. There are various other unidentified medicinal plants which may have antivenin activity or supplement the action of anti-snake venom. Therefore, investigation of pharmacological potential and evaluation of antivenin properties of existing plants is important through well designed and validated scientific studies. Besides, developing countries should

intensify their efforts in documenting the ethnomedical data and scientific research on medicinal plants including the initial vital leads provided by ethnic group who has predominantly used the particular medicinal plant. This ethnopharmacological approach will help people to get better informed about effective herbal remedies available and increase their social responsibility for cultivation of eco-friendly herbs.

Conclusion

Snakebite is a preventable and treatable disease. There is a strong need for development of strategies for prevention and control of snake envenomation. The primary emphasis should be given for educating the healthcare workers, students, communities and others. Governing bodies should take initiatives to support snake-bite management strategies and their successful implementation. Low cost, broadly neutralizing recombinant antivenoms are required to be developed which can be used against multiple species. Similarly, newer technologies like proteomic, transcriptomic and genomic can be targeted. Moreover, natural biodiversity and availability of invaluable plant resources can contribute for development of new drug molecules or adjuvant therapies for existing snakebite treatment. Traditional knowledge and controlled clinical trials data are necessary to demonstrate the pharmacokinetics, efficacy and safety of plant extract or its bioactive compounds. These strategies will certainly help in improving the health status of society in snakebite and several other pathophysiological conditions.

Conflict of interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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