Indian Journal of Experimental Biology
Vol. 48, September 2010, pp. 865-878

Review Article

Herbs and herbal constituents active against snake bite

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Snake bite, a major socio-medical problem of south east asian countries is still depending on the usage of antisera as the one and only source of treatment, which has its own limitations. In India, mostly in rural areas, health centres are inadequate and the snake bite victims mostly depend on traditional healers and herbal antidotes, as an alternative treatment. The present review has been focussed on the varied folk and traditional herbs and their antisnake venom compounds, which might be a stepping stone in establishing the future therapy against snake bite treatment and management.

Keywords: Alternative medicines, Herbal compound, Snake bite, Snake bite treatment, Snake venom

Introduction

Snake bite, till date remains a public health hazard in tropical countries, especially in India. Accurate records to determine the exact epidemiology or even mortality in snake envenomation cases are inadequately available[1]. Hospital records fall far short of the actual number owing to the dependence on traditional healers and practitioners. In India, on an average 2,50,000 snake bites are recorded in a single year. There are 52 poisonous species of snakes available in India, of which majority of the bites and mortality are attributed to species like *Ophiophagus hannah* (king cobra), *Naja naja* (spectacled cobra), *Daboia russelli* (Russell's viper), *Bungarus caeruleus* (common krait) and *Echis carinatus* (saw-scaled viper).

Snake venom, the most complex of all poisons is a mixture of enzymatic and non-enzymatic toxic compounds as well as other non-toxic proteins, non-proteins, including carbohydrates and metals that is stored in poison glands. There are more than twenty different enzymes including procoagulant enzymes, haemorrhagins, cytolytic or necrotic toxins, pre-synaptic and post-synaptic neurotoxins, phospholipases A2, B, C, D, hydrolases, phosphatases (acid and alkaline), proteases, esterases, acetylcholinesterase, transaminase, hyaluronidase, phosphodiesterase, nucleotidase, ATPase and nucleosidases (DNA and RNA). Very few non-protein components have been isolated from snake venom, an anticonvulsant non-protein cardiotoxin is among them[2]. The pathophysiologic basis for morbidity and mortality is the disruption of normal cellular functions by these enzymes and toxins.

The treatment for snake bite is as variable as the bite itself. The only available treatment is the usage of antivenom against snake bite. The first antivenom (called an anti-ophidic serum) was developed by Albert Calmette, a French scientist of the Pasteur Institute in 1895, against the Indian Cobra (*Naja naja*). Antivenom binds to and neutralizes the venom, stopping further damage, but do not reverses the damage already done. Some individuals may react to the antivenom with an immediate hypersensitivity reaction[3]. Other alternative treatment involves the usage of folk and traditional medicines in snake bites. Medicinal herbs are the local heritage with global importance. Various plants have been used against snake bite, in folk and traditional medicine. In Ayurvedic system of medicine different plants and their compounds are reported to possess antisnake venom activity. But they also possess their individual toxicities and most of the folk medicinal plants have no scientific validation. This review is an attempt to focus on the antivenom treatment of snake bite, its limitations, herbal antagonists and herbal constituents active against snake bite and its future.

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Antivenom treatment of snake envenomation and its limitations

The most common and effective method of treating snake bite victims is through antivenom, a serum made from the venom of the snake[4]. In India, polyvalent antivenom is prepared by Central Research Institute, Kasauli, Simla, and the Haffkine Corporation, Parel, Mumbai. The WHO has designated the Liverpool School of Tropical Medicine as the international collaborating centre for antivenom production and/or testing[4]. Antivenoms, in most countries are costly and may be in limited supply. Antivenoms for therapeutic use are often preserved as freeze-dried ampoules, but some are available only in liquid form and must be kept refrigerated. The majority of snake antivenoms are administered intravenously. The intramuscular route has been questioned in some situations as they are not uniformly effective. Antivenom should be given as quickly as possible so that the venom's side effects can be managed. Antivenom should be given only if the range of specificity is stated which includes the species known or thought to have been responsible for the bite. Liquid antivenom that turned opaque should not be used because precipitation of protein indicates loss of activity which is directly proportional to increased risk of reactions.

In India, other centres which are involved in manufacturing of antivenom are Bharat Serums and Vaccines Ltd., Mumbai, Serum Institute, Pune, King Institute, Chennai, Vins Bio-products Ltd.; and Biological 'E' Ltd., Hyderabad, *etc.* Antivenom serum (AVS) manufacturers recommend skin sensitivity testing to predict adverse AVS reactions. But, the usefulness of skin testing is doubtful, as skin testing carries the risk of inducing an acute reaction and delays the initiation of AVS administration.

Herbal antidotes active against snake bite

In almost all parts of the world, where venomous snakes occur, numerous plant species are used as folk medicine to treat snake bite. Generally an aqueous, methanol or ethanol extract is prepared out of the plant parts. Topical application of the plant or its sap onto the bitten area, chewing leaves or barks or drinking plant extracts or decoctions or injecting the extrcts are some procedures intended to counteract snake venom activity.

The roots of the plant *Ophiorrhiza mungo, Peristrophe bicalyculata, Gymnema sylvestre Gloriosa superba, Cucumis colosynthis, Alangium salvifolium,* leaves of *Enicostemma axillare, Calycopteris floribunda, Calotropis gigantea, Aristolochia indica* are used in Ayurvedic medicine. Ayurveda states the usage of specific plants against specific snake bites, e.g. root extract of *Abrus precatorius* is used against krait bite, leaf paste of *Azadirachta indica* with rock salt is used against viper bites. Leaves and bark of *Casearia sylvestris,* (guacotonga) are used as a standard Ayurvedic drug to treat snake bite in Columbia, India, etc. *Aristolochia indica* is used as a decoction for snake bite. Seeds of *Psoralea corylifolia* are used both in Ayurveda and Siddha against snake bite. *Origanum dictamnus* juice is consumed in wine to cure snake bite. Tea made from the leaves of *Cecropia peltata* is used as a cure for a wide variety of ailments including snake bite. *Achyranthes aspera*, is used in treatment of bleeding, renal complications, scorpion bite, snake bite, *etc*.

The first scientific investigation regarding the herbal antidotes was from Knowles[5]. He screened several plants/plant constituents, used by local healers, but failed to report their efficacies against snake envenomation, either due to sublethal dose of venom or non lethal dose. Later, Mhaskar and Caius[6] challenged the effectiveness of herbal antidotes by screening 314 plants and 184 combinations against venom induced lethality, ignoring the systemic changes induced by snake venom[6]. This pioneering theory was later contradicted by various reports on effectiveness of herbal antidotes against systemic toxicities as well as lethality. The ether soluble fraction of *Aristolochia* species, inactivates *Naja naja* venom and reduces haemorrhage caused by *Trimeresurus flavoviridis* and *Vipera russellii* venoms[7,8]. *Eclipta prostrata* L. (Asteraceae) is used as an anti-venom against snake bite in China and in Brazil. *Schumanniophyton magnificum, Eclipta prostrata* or *Aristolochia shimadai*, have the capacity to inhibit phospholipase A2, other enzymes (e.g. ATPase) along with other physiological and biochemical properties (such as effects on uterine tone or the protection of mitochondrial membranes). Antihaemorrhagic effect of persimmon tannin from *Diospyros kaki* is also well known. The survival time was prolonged after pretreatment with extracts of *Diodia scandens* and *Andrographis paniculata*[9].

Rhizomes of Curcuma Sp. inactivated postsynaptic neurotoxin of the Thai cobra (*Naja naja siamensis*) in mice[10]. Aqueous extracts of the bark of *Schumanniophyton magnificum* and the leaves of *Mucuna pruriens var. utilis*, *Strophanthus gratus* and *Strophanthus hispidus* have the ability to prolong the clotting time when administered along with a standardised dose of *Echis carinatus* venom[11]. The survival time of male abino mice was increased by the extract of the leaves of *Guiera senegalensis* when compared to the *Echis carinatus* and *Naja nigricollis* venom treated animals[12]. *Bothrops atrox* venom induced haemorrhage was completely neutralized by the extracts of the stem barks of *Brownea rosademonte, Tabebuia rosea,* the whole plants of *Pleopeltis percussa, Trichomanes elegans,* rhizomes of *Heliconia curtispatha,* leaves and branches of *Bixa orellena, Phylodendrum tripartitum, Struthanthus orbicularis, Gozalagunia panamensis,* the ripe fruit of *Citrus limon* leaves, branches and stems of *Ficus nymphaeifolia*[13]. Partial protection of haemorrhage was also shown by *Aristolochia grandiflora, Columnea kalbreyeriana, Sida acuta, Selaginella sp., Pseudele- phantopus spicatus*, rhizomes of *Renealmia alpinia*, stem of *Strychnos xynguensis*, leaves, branches and stem of *Hyptis capitata, Ipomoea cairica, Neurolaena lobata, Ocimum micranthum, Piper pulchrum, Siparuna thecaphora, Castilla elastica, Allamanda cathartica,* the macerated fruits of *Capsicum frutescens*, unripe fruits of *Crescentia cujete,* leaves and branches of *Piper arboretum* and *Passiflora quadrangularis*[13]. The stem barks of *Brownea rosademonte, Tabebuia rosea*; rhizomes of *Renealmia alpinia, Heliconia curtispatha*; the whole plants of *Pleopeltis percussa, Trichomanes elegans*; and the ripe fruits of *Citrus limon*, demonstrated 100% neutralizing capacity of snake (*Bothrops atrox*) venom within 48 hours. Partial protection was also shown by the leaves, branches and stem of *Costus lasius*; the whole plant of *Sida acuta*; rhizomes of *Dracontium croatii*; leaves and branches of *Bixa orellana* and *Struthanthus orbicularis*[14]. *Bothrops atrox* venom induced haemorrhage was completely neutralized by stem barks of *Brownea rosademonte* and *Tabebuia rosea*; the whole plants of *Pleopeltis percussa, Trichomanes elegans* and *Senna dariensis*; rhizomes of *Heliconia curtispatha*; leaves and branches of *Bixa orellana, Philodendron tripartitum, Struthanthus orbicularis* and *Gonzalagunia panamensis*; ripe fruits of *Citrus limon*; leaves, branches and stem of *Ficus nymphaeifolia* and moderate neutralization were shown by the whole plants of *Aristolochia grandiflora*, *Columnea kalbreyeriana, Sida acuta, Selaginella articulata* and *Pseudoelephantopus spicatus*; rhizomes of *Renealmia alpinia*; the stem of *Strychnos xinguensis*; leaves, branches and stems of *Hyptis capitata, Ipomoea cairica, Neurolaena lobata, Ocimum micranthum, Piper pulchrum, Siparuna thecaphora, Castilla elastica and Allamanda cathartica*; the macerated ripe fruits of *Capsicum frutescens*; the unripe fruits of *Crescentia cujete*; leaves and branches of *Piper arboreum* and *Passiflora quadrangularis*[15]. *Mucuna pruriens var. utilis* seed aqueous extract significantly inhibited the *Echis carinatus* venom induced myotoxic, cytotoxic and coagulation activities in experimental animals[16]. Aqueous and alcoholic extracts of dried roots of *Mimosa pudica* inhibited lethality, myotoxicity and toxic enzymes of *Naja kaouthia* venom[17]. Oral administration of garlic could be used as a prophylactic tool against cobra venom induced histological and histochemical patterns of the gastric and hepatic tissue changes in rats[18]. A water-methanol extract of *Parkia biglobosa* stem bark extract could neutralize two snake venoms (*Naja nigricollis*, and *Echis ocellatus*) in several experimental models[19]. Phospholipase activity of *Crotalus durissus terrificus* venom and only partial inhibition of Bothrops venoms was shown by crude aqueous extract of *Mandevilla velutina*[20]. The extract of the Brazilian plant *Marsypianthes chamaedrys* inhibited fibrinogen clotting induced by several Brazilian snake venoms, indicating that it affects thrombin-like enzymes[21]. Aqueous extract of *Casearia sylvestris* showed anti PLA2, hemorrhagic and myotoxic activities caused by crude snake venoms and toxins[22]. On the other hand, *Casearia mariquitensis* inhibited some hematological and systemic alterations induced by *Bothrops neuwiedi pauloensis* venom[23]. *Mandevilla illustris* inhibited phospholipase activity of *Crotalus durissus terrificus* snake venom along with prolongation of the survival time and a decrease in lethality[24]. *Mimosa pudica* has been reported to possess anti-hyaluronidase activity against *Naja naja, Vipera russelli* and *Echis carinatus* venom[25]. The butanolic extract of *Mimosa pudica* and *Eclipta prostrata* partially inhibited the hemorrhagic activity but displayed very low anti-phospholipase A2 activity and did not inhibit proteolytic activity of Malayan pit

viper venom[26]. Edema, defibrination and coagulation effects of *Bothrops asper* venom were neutralized by the leaves and branches of *Bixa orellen, Ficus nymphaeifolia, Struthanthus orbicularis* and *Gonzalagunia panamensis*; the stem barks of *Brownea rosademonte* and *Tabebuia rosea*; the whole plant of *Pleopeltis percussa* and *Trichomanes elegans*; rhizomes of *Renealmia alpinia*, *Heliconia curtispatha* and *Dracontium croatii*, and the ripe fruit of *Citrus limon*[27]. The ethanol root extract of *Acalypha indica* possesses potent snake venom neutralizing properties[28]. Aqueous extract of *Tabernaemo ntana catharinensis* inhibited the lethal activity of *Crotalus durissus terrificus* snake venom[29] and partially neutralized myotoxic effect of *Bothrops jararacussu* venom and two of its myotoxins [bothropstoxin-I (BthTX-I), catalytically inactive, and II (BthTX-II), showing low PLA2 activity][30]. The methanol extract of the root bark of *Annona senegalensis* Pers caused reduction in the *Naja nigricotlis nigricotlis* venom induced hyperthermia in rats[31]. *Musa paradisiaca* L. successfully neutralized viper venom actions in *in vitro* experiments only[32]. *Pentaclethra macroloba* exhibited full inhibition of hemorrhagic and nucleolytic activities induced by several snake venoms, along with partial inhibition of myotoxic, lethal, phospholipase and edema activities. It totally inhibited *Bothrops jararacussu* metalloprotease induced hemorrhage in *in vivo* model[33]. Aqueous extracts of *Croton urucurana* antagonized the hemorrhagic activity of *Bothrops jararaca* venom and proanthocyanidins were involved in this activity[34]. Aqueous extracts of fresh roots, stems or leaves of *Mikania glomerata*, efficiently neutralized different toxic, pharmacological, and enzymatic effects induced by venoms from Bothrops and Crotalus snakes[35]. *Cordia verbenacae* inhibited paw edema induced by *Bothrops jararacussu* snake venom[36]. Aqueous extract from aerial parts of *Bauhinia forficata* is a promising source of natural inhibitors of serine-proteases involved in blood clotting disturbances induced by Bothrops and Crotalus crude venoms[37]. The methanol bulb extract of *Crinum jagus* significantly protected mice from death, myonecrosis and haemorrhage induced by the lethal effects of *Echis ocellatus, Bitis arietans* and *Naja nigricollis* venoms[38]. Tamarind seed extract inhibited the PLA2, protease, hyaluronidase, l-amino acid oxidase and 5'-nucleotidase enzyme (major hydrolytic enzymes) activities of *Vipera russelli* venom in a dose-dependent manner. Further, the extract neutralized the degradation of the B-beta chain of human fibrinogen and indirect hemolysis caused by venom. The extract exerted a moderate effect on the clotting time. Edema, hemorrhage and myotoxic effects along with lethality, induced by venom were neutralized significantly when different doses of the extract were preincubated with venom before the assays. On the other hand, animals that received extract 10 minutes after the injection of venom were protected from venom induced toxicity[39]. Dichloromethane extract of leaves of *Artemisia campestris* L neutralized the venom induced actions of viper *Macrovipera lebetina*[40]. Ethanol extract of leaves of *Galactia glaucescens* prevented the neuromuscular paralysis induced by *Crotalus durissus terrificus* venom[41]. Oedema, haemorrhage, myonecrosis and coagulation induced by Indian *Echis carinatus* (saw-scaled viper) venom were neutralized by the methanol seed extract of *Vitis vinifera* L[42]. The aqueous extract of *Schizolobium parahyba* showed potent antisnake venom activity[43,44]. The active fractions of *Aristolochia indica, Hemidesmus indicus, Strychnos nux vomica, Gloriosa superba, Eclipta prostrata,* and *Andrographis paniculata* neutralized rattle snake venom induced actions[45]. The animals that received orally the extract of *Aristolochia odoratissima* leaves were protected against *Bothrops atrox* venom as mortality of experimental animals decreased from 100 to 80%[46].

The methanol root extracts of *Vitex negundo* Linn. and *Emblica officinalis* significantly neutralized the *Vipera russelli* and *Naja kaouthia* venom induced lethal activity *in vivo* studies; *Vipera russelli* venom-induced haemorrhage, coagulant, defibrinogenating and inflammatory activity were significantly neutralized by both plant extracts[47]. *Hemidesmus indicus* root extracts effectively neutralized Viper venom-induced lethal, haemorrhagic, coagulant, anticoagulant and inflammatory activities[48]. An active compound from the *Strychnus nux vomica* whole seed extract, neutralised *Daboia russelli* venom induced lethality, haemorrhage, defibrinogenating, PLA2 enzyme activity and *Naja kaouthia* venom induced lethality, cardiotoxicity, neurotoxicity, PLA2 enzyme activity, and it also inhibited viper venom induced lipid peroxidation in experimental animals[49] (Table 1).

Table 1—List of plants used against snake envenomation

Scientific name	Parts of the plant used	Scientific name	Parts of the plant used
Abrus precatorius	roots	*Echinacea angustifolia*	
Acalypha indica	roots	*Echinacea pallida*	
Achyranthes aspera		*Echinacea purpurea*	
Acorus calamus Linn	rhizomes	*Eclipta prostrata* L.	
Actaea racemosa		*Emblica officinalis*	roots
Alangium salvifolium	roots	*Enicostemma axillare*	leaves
Allamanda cathartica	leaves, branches, stem	*Equisetum giganteum*	rhizomes
Aloe barbadensis	leaves	*Ficus lacor*	latex
Andrographis piniculata		*Ficus nymphaeifolia*	leaves, branches, shoot
Annona senegalensis Pers	root, bark	*Ficus religiosa*	flower
Antidesma bunius Linn.	leaves	*Galactia glauscescens*	leaves
Arctium lappa		*Gonzalagunia panamensis*	leaves, branches
Aristolochia grandiflora	whole plant	*Guiera senegalensis*	leaves
Aristolochia odoratissima	leaves	*Gymnema sylvestre*	roots
Aristolochia shimadai		*Heliconia curtispatha*	rhizomes
Artemisia campestris L	leaves	*Hemidesmus indicus*	roots
Azadirachta indica	leaves	*Hyptis capitata*	leaves, branches, stem
Biophytum sensitivum	whole plant	*Impatiens balsamina*	flower
Bixa orellana	leaves, branches, shoot	*Ipomoea cairica*	leaves, branches, stem
Bombax ceiba Linn	shoot, leaves	*Leucas linifolia*	
Brownea rosa de monte	stem, barks	*Lysimachia nummularia* L.	
Buchnania lanzan Spr	stem, bark	*Mandevilla illustris*	
Calotropis gigantea	leaves, roots	*Marsypianthes chamaedrys*	
Calycopteris floribunda	leaves	*Melianthus major*	flower
Capsicum frutescens	fruit	*Mikania glomerata*	roots, stems, leaves
Casearia mariquitensis		*Mimosa pudica*	roots, whole plant
Casearia sylvestris	leaves & bark	*Moringa oleifera* Lamk	shoot, leaves
Cassia occidentalis	roots	*Mucuna pruriens var. utilis*	seeds
Castilla elastica	leaves, branches, stem	*Mucuna pruriens var. utilis*	leaves
Cecropia peltata	leaves	*Musa paradisiaca*	
Cinnamomum zeylanicum		*Nerium indicum* Mill	leaves
Citrus limon	fruit	*Nerium oleander*	
Columnea kalbreyeriana		*Neurolaena lobata*	leaves, branches, stem
Cordia verbenacae		*Ocimum micranthum*	leaves, branches, stem
Costus lasius	leaves, branches, stem	*Oldenlandia corymbosa* L	
Crescentia cujete	fruit	*Ophiorrhiza mango*	roots
Crinum jagus	bulb	*Origanum dictamnu*	seeds
Croton urucurana		*Papever somniferum*	
Cucumis colocynthis	roots	*Paris polyphylla*	roots
Curcuma longa	rhizomes	*Parkia biglobosa*	
Diodia scandens		*Passiflora quadrangularis*	leaves, branches
Diospyros kaki		*Pentaclethra macroloba*	
Dracontium croatii	rhizomes	*Peristrophe bicalyculata*	roots
Sida acuta	whole plant	*Siparuna thecaphora*	leaves, branches, stem
Philodendron tripartitum	shoot, leaves	*Strophanthus gratus*	leaves
Piper arboretum	leaves, branches	*Strophanthus hispidus*	leaves
Piper pulchrum	leaves, branches, stem	*Struthanthus orbicularis*	leaves, branches, shoot
Pleopeltis percussa	whole plant	*Strychnos nux vomica*	seeds
Prenanthes alba	leaves	*Strychnos xinguensis*	stem, leaves, branches
Pseudelephantopus spicatus	whole plant	*Tabebuia avellanedae*	bark
Psoralea corylifolia	seeds	*Tabebuia rosea*	stem barks
Raphanus sativus	tuber	*Tabernaemontana catharinensis*	
Rauwolfia serpentine	whole plant	*Tamarindus indica*	
Renealmia alpinia	rhizomes	*Trichomanes elegans*	whole plant
Rhizoma paridis	roots	*Ulmus rubra*	

Table 1—List of plants used against snake envenomation—Contd.

Scientific name	Parts of the plant used
Schizolobium parahyba	leaves
Schumanniophyton magnificum	bark
Selaginella articulata	whole plant
Senna dariensisida acuta	whole plant
Vitex negundo Linn	roots
Vitis vinifera L	seeds
Woofordia fruitcosa	
Xanthium sibiricum	leaves

Herbal constituents active against snake envenomation

Acids—Aristolochic acid, contained in *Aristolochia* produces increase in immune response and it also inhibits the lytic activity and the edematose properties of some phospholipases of snake venoms[50]. 2-OH-4-methoxy benzoic acid, isolated and purified from *Hemidesmus indicus,* possessed potent antiinflammatory, antipyretic and antioxidant properties of viper venom[51]. Lethality induced by Viper venom was maximally neutralized with 2-hydroxy-4-methoxy benzoic acid and anisic acid, both in *in vitro* and *in vivo* studies. The compound 2-OH-4-methoxy benzoic acid also showed adjuvant effects and antiserum action potentiation against *Vipera russelli* venom[52]. The exact mechanisms of venom neutralization were not established, except for 2-hydroxy-4-methoxy benzoic acid, where the functional groups, methoxy and hydroxy were partly responsible for the neutralization of the lethal effect and haemorrhagic activity[52]. The venoms of common Indian snakes *Vipera russelli, Echis carinatus, Naja kaouthia* and *Ophiophagus hannah* were taken to evaluate the lethal, haemorrhagic and defibrinogenation action neutralization with four compounds (2-hydroxy-4-methoxy benzoic acid from *Hemidesmus indicus*, anisic acid from *Pimpinella anisum*, salicylic acid from *Filipendula ulmaria, Salix alba* and aspirin from *Salix alba*) in experimental animals. Lethal action of venom were maximally neutralized with 2-hydroxy-4-methoxy benzoic acid and anisic acid, both in *in vitro* and *in vivo* studies. Haemorrhagic activity of Viper and Echis venom was neutralized with salicylic acid[53]. Rosmarinic acid, a new antidote against snake (*Bothrops jararacussu*) venom, was isolated which possessed phospholipase A2 (from *Cordia verbenacea)* inhibitor activity; it also inhibited most of the myotoxic activity with partial inhibition of edema along with the ability to potentiate commercial equine polyvalent antivenom in neutralizing lethal and myotoxic effects of the crude venom and of isolated PLA2s in experimental models.

Alkaloids—Atropine is found in some of the Solanacae family members and it exerted inhibitory action against the venom of green and black mamba (*Dendroaspis angusticeps* and *D. polylepsis*); these venoms are mainly responsible for neuro-transmitter release at cholinergic nerve terminals, therefore it was suspected that a cholinergic blocker like atropine may reduce their effects. AIPLAI (*Azadirachta indica* PLA2 inhibitor) was purified from the methanol leaf extract of *Azadirachta indica* (Neem); it inhibited the cobra and *Russell's viper* venoms (RVVs) phospholipase A2 enzymes in a dose-dependent manner. AIPLAI significantly inhibited PLA2 enzymes, higher in cobra venom (*Naja naja* and *Naja kaouthia*) compared to that of crude RVV (*Daboia russelli*) when tested under the same condition[54].

Coumestan and steroids—Viper and cobra venom neutralization was shown by beta sitosterol and stigmasterol, isolated from the methanol root extract of *Pluchea indica*[55]. The active fraction could also antagonize venom-induced changes in lipid peroxidation and superoxide dismutase activity. Wedelolactone has been identified as a coumestane contained in *Eclipta prostrata* L. and was suggested to be an active component in fighting against snake venoms[56]. Wedelolactone, sitosterol and stigmasterol inhibits the effect of South American rattle snake[57].

Enzymes, peptides and pigments—Snake venom molecule are composed of three-dimensional proteins and some non-protein components. These proteins could be dissolved with natural solvents like bromelain and papain. Bromelain is found in pineapple (*Ananas comosus*) and papain is present in papaya fruit (*Carica papaya*). Thus these two natural proteolytic enzymes could be used to neutralize snake venom proteins. A peptide compound with a molecular weight of 6000 Da, reported to possess anticardiotoxic activity against cobra venom was isolated from the plant *Schumanniphyton magnificum*[58]. Turmerin, a protein from turmeric (*Curcuma longa* L.) inhibits the enzymatic activity and neutralizes cytotoxicity, oedema and myotoxicity of multitoxic phospholipase A2 of cobra (*Naja naja*)[59]. Melanin extracted from black tea was reported for the first time to possess antivenom activity against *Agkistrodon contortrix laticinctus, Agkistrodon halys blomhoffii* and *Crotalus atrox* snake venoms[60].

Glycoprotein and glycosides—A multiform glycoprotein (whose oligosaccharide chains were functional) was isolated from *Mucuna pruriens* seeds, neutralized *Echis carinatus* venom induced actions[61]. A glycoprotein, (WSG) was isolated from a folk medicinal plant *Withania somnifera*[62]. The WSG inhibited the phospholipase A2 activity of NN-XIa-PLA2 isolated from the cobra venom (*Naja naja*), completely at a mole-to-mole ratio of 1:2 (NN-XIa-PLA2: WSG)[62] 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 WSG also inhibited several other PLA2 isoforms from the venom to varying extent. Hyaluronidase activity of cobra (*Naja naja*) and viper (*Daboia russelli*) venoms were inhibited by WSG. It also inhibited hyaluronidase activity of Indian cobra (*Naja naja*) venom[63,64]. Benzoylsalireposide and salireposide isolated from *Symplocos racemosa* inhibited phosphodiesterase I activity against snake venom. The methanol extract of the stem bark of *Schumanniophyton magnificum* and schumanniofoside, a chromone alkaloidal glycoside was isolated which reduced the lethal effect of black cobra (*Naja melanoleuca*) venom in mice. This effect was maximum when the venom was mixed and incubated with the extract or schumanniofoside. It is thought that the mode of action is by oxidative inactivation of the venom.

Phenols—PLA2 activity of *Bothrpos asper* venom was neutralized by 4-nerolidylcatechol isolated from *Piper umbellatum* and *Piper peltatum*[65]. The ethanol extract from seed kernels of Thai mango (*Mangifera indica* L.) and its major phenolic principle (pentagalloyl glucopyranose) exhibited dose-dependent inhibitory effects on phospholipase A2, hyaluronidase and L-amino acid oxidase of *Calloselasma rhodostoma* and *Naja naja kaouthia* venoms in *in vitro* tests. The anti-hemorrhagic and anti-dermonecrotic activities of seed kernal against both venoms were clearly supported by *in vivo* tests[66]. The plant polyphenols from the aqueous extracts of *Pentace burmanica*, *Pithecellobium dulce*, *Areca catechu* and *Quercus infectoria* block non-selectively the nicotinic acetylcholine receptor by precipitation of *Naja kaouthia* venom[67].

Pterocarpanes—The aqueous alcohol extract of the root of a South-America plant called *Cabeca de Negra* is used against snake venom. From this source, cabenegrin A-I and cabenegrin A-II were isolated, which have been found to be an anti-snake oral antidote[68]. Similarly the extract of a South American plant, *Harpalyce brasiliana* Benth, commonly called Portuguese Snake Herb, also yielded cabenegrin A-II (phenolic pterocarpan in nature). Edunol, a pterocarpan isolated from *Harpalyce brasiliana*, used in the northeast of Brazil against snake bites and roots of two Mexican 'snakeweeds', *Brongniartia podalyrioides* and *Brongniartia intermedia* (Leguminosae), reduced the expected mortality of mice previously treated with *Bothrops atrox*[69] venom along with antimyotoxic, antiproteolytic and PLA2 inhibitor properties[70].

Tannins—The tannin from persimmon, a fruit from *Diospyrus kaki* inhibits edema in mice, induced by sea snake and also improved the survival rate in mice injected with *Laticauda semifasciata* and *Trimeresurus flavoviridis* venom[71]. Ellagic acid, a compound isolated from the aqueous extract of *Casearia sylvestris* has been reported to possess anti snake venom activity, mainly against Bothrops genus[72].

Terpenoids—Glycyrrhizin, a natural triterpenoid saponin extracted from the root of *Glycyrrhiza glabra* (licorice), with a molecular mass of 840 Da, has been characterized as a thrombin inhibitor[73]. This compound is known for its anti-inflammatory activity and Glycyrrhizin also exhibits *in vivo* antithrombotic properties against snake venom; it prevents both *in vitro* and *in vivo* venom-induced changes in hemostasis, suggesting a potential antiophidic activity[74]. A potassium salt of gymnemic acid, which is a triterpenoid glycoside obtained from *Gymnema sylvestre* inhibits ATPase in *Naja Naja* venom[75,76]. 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 russelli* venom. It also neutralized *Naja kaouthia* venom induced lethality, cardiotoxicity, neurotoxicity and respiratory changes in experimental animals[77]. *Bothrops neuwiedi* and *Bothrops jararacussu* venom induced hemorrhagic, fibrinogenolytic and caseinolytic activities of class P-I and III metalloproteases were neutralized by neo-clerodane diterpenoid, isolated from *Baccharis trimera*[78]. Oleanolic acid inhibited sPLA (2) activities of *Vipera russelli* and *Naja naja* snake venoms in a concentration-dependent manner. Inhibition of *in vitro* and *in vivo* sPLA2 activity by oleanolic acid

explains the observed anti-inflammatory properties of several oleanolic acid-containing medicinal plants[79]. The pentacyclic triterpenes (free or as glycosides) are found widely in several antisnake venom plants (*Aegle marmelos*, *Centipeda minima*, *Aloe barbadensis*, *Phyllanthus niruri*, *Alstonia scholaris*, *Phyllanthus emblica*, *Elephantopus scaber*, etc.) and provide nearly 20% protection against snake venom[80]. Quinovic acid-3-O-alpha-L-rhamnopyranoside[81], quinovic acid-3-O-beta-D-fucopyranoside, and quinovic acid-3-O-beta-D-glucopyranosyl (1-->4)-beta-D-fucopyranoside isolated from the ethyl acetate extract of *Bridelia ndellensis* barks and *Mitragyna stipulosa* showed significant inhibitory activity against snake venom phospho-diesterase-I[82,83]. Triterpenoid saponin from *Pentaclethra macroloba,* inhibited antiproteolytic and anti-hemorrhagic actions induced by Bothrops snake venoms. These inhibitors were able to neutralize the hemorrhagic, fibrin(ogen)olytic, and proteolytic activities of class P-I and P-III metalloproteases isolated from *Bothrops neuwiedi* and *Bothrops jararacussu* venoms[84]. Ursolic acid a common constituent of many medicinal plants, inhibited PLA2 enzymes purified from *Vipera russelli*, *Naja naja* venom[85].

Quinonoid xanthene—Ehretianone, a quinonoid xanthene isolated from the root bark of *Ehretia buxifolia*. Roxb. has been reported to possess anti snake (*Echis carinatus*) venom activity[86].

Resveratrol—Hong Bei Si Chou is a herbal medicine used to treat snake bite in Guangxi province of China. It was found that resveratrol (3,4',5-trihydroxytransstilbene) isolated from the ethyl acetate part of Hong Bei Si Chou could antagonize snake toxins both *in vivo* and *in vitro*[87]. Alkaloid (12-methoxy-4-methylvoachalotine) extract of *Tabernaemontana catharinensis* inhibited lethality induced by *Crotalus durissus terrificus* snake venom[88].

Miscellaneous chemical groups and compounds—Several plant constituents like flavonoids, quinonoid, xanthene, polyphenols and terpenoids possesses protein binding and enzyme inhibiting properties and also inhibit snake venom phospholipase A2 (PLA2) activities of both Viper and Cobra venom[89]. Total inhibition of hemorrhage was observed with the ethanol, ethyl acetate and aqueous extracts of *Bursera simaruba, Clusia torresii, Clusia palmana, Croton draco, Persea americana, Phoebe brenesii, Pimenta dioica, Sapindus saponaria, Smilax cuculmeca* and *Virola koschnyi*[83]. Chemical analysis of these extracts identified catequines, flavones, anthocyanines and condensated tannins, which may be responsible for the inhibitory effect observed, probably owing to the chelation of the zinc required for the catalytic activity of *Bothrops asper* venom's hemorrhagic metalloproteinases. Plant-derived, aristolochic acid, indomethacin, quercetin, curcumin, tannic acid, and flavone exhibited inhibition, with aristolochic acid and quercetin completely inhibited the hyaluronidase enzyme activity[90]. Further, these inhibitors not only reduce the local tissue damage but also retard the easy diffusion of systemic toxins and hence increase survival time. Medicinally important herbal compounds (acalyphin, chlorogenic acid, stigmasterol, curcumin and tectoridin) were screened against Russell's viper PLA2[91]. These compounds showed favorable interactions with the amino acid residues at the active site of Russell's viper PLA2, thereby substantiating their proven efficacy as anti-inflammatory compounds and as antidotes. An active compound (SNVNF) was isolated and purified from the whole seed extract of *Strychnos nux vomica*, which could effectively antagonise *Daboia russelli* venom induced lethality, haemorrhage, defibrinogenating, oedema, PLA2 enzyme activities and *Naja kaouthia* induced lethality, cardiotoxicity, neurotoxicity, PLA2 enzyme activities. Hexane extract of *Curcuma longa* rhizomes, ar-turmerone[92] also inhibited the proliferation and the natural killer cell activity of human lymphocytes. This compound has anti lethal activity against venom of *Crotalus durissimus terrificus*. Moreover when it was injected in mice it showed anti- hemorrhagic activity against *Bothrops jararaca* venom. (Table 2).

Mechanism of snake venom neutralization by herbal compounds

Herbal compounds that possess snake venom neutralization properties in experimental animal models (*in vivo* and *in vitro*) usually follows three protocols—(1) venom- herbal compounds mixed together, (2) herbal compounds followed by venom, and (3) venom followed by herbal compounds. Among these, the third technique is similar to clinical conditions. The venom dose is one of the critical factors, on which the herbal compounds could show their neutralizing effects. Higher the venom dose, less the fold of neutralization. So, what is desirable is that the venom should be tried from lower to higher dose. For this, a huge number of animals are required, which is sometimes very difficult from animal ethical issues.

Table 2—List of herbal compounds active against snake envenomation

Compound	Plant source	Reference
Acids		
Aristolochic acid	Aristolochia sp.	50
2-OH-4-methoxy benzoic acid	*Hemidesmus indicus*	51, 52
2-hydroxy-4-methoxy benzoic, anisic acid, salicylic acid, aspirin	*Hemidesmus indicus, Pimpinella anisum, Filipendula ulmaria, Salix alba*	53
Rosmarinic acid	*Cordia verbenacea*	
Alkaloids		
Atropine	*Dendroaspis angusticeps* and *D. polylepsis*	
AIPLAI (*Azadirachta indica* PLA2 inhibitor)	*Azadirachta indica*	54
Coumestan and Steroids		
Beta sitosterol and stigmasterol	*Pluchea indica*	55,57
Wedelolactone	*Eclipta prostrata* L.	56,57
Enzymes, Peptides and Pigments		
Bromelain	*Ananas comosus*	
Papain	papaya	
Peptide	*Schumanniphyton magnificum*	58
Turmerin	*Curcuma longa* L	59
Melanin	Black Tea	60
Glycoprotein and Glycosides		
Glycoprotein	*Mucuna pruriens*	61
WSG	*Withania somnifera*	62, 63
Benzoylsalireposide and salireposide	*Symplocos racemosa*	64
Phenols		
4-nerolidylcatechol	*Piper umbellatum* and *Piper peltatum*	65
Pentagalloyl glucopyranose	*Mangifera indica* L	66
polyphenols	*Pentace burmanica*, *Pithecellobium dulce*, *Areca catechu* and *Quercus infectoria*	67
Pterocarpanes		
Cabenegrin A-I and cabenegrin A-II	*Cabeca de negro*	68
Edunol	*Harpalyce brasiliana*	69, 70
Tannins		
Tannin	*Diospyrus kaki*	71
Ellagic acid	*Casearia sylvestris*	72
Terpeinoids		
Glycyrrhizin	*Glycyrrhiza glabra*	73, 74
Potassium salt of gymnenic acid	*Gymnema sylvestre*	75, 76
Lupeol acetate	*Hemidesmus indicus* R.Br	77
Neo-clerodane	*Baccharis trimera*	78
Oleanolic acid		79
Pentacyclic triterpenes	*Aegle marmelos*, *Centipeda minima*, *Aloe barbadensis*, *Phyllanthus niruri*, *Alstonia scholaris*, *Phyllanthus emblica*, *Elephentopus scaber*	80
Quinovic acid-3-O-beta-D-fucopyranoside, and quinovic acid-3-O-beta-D-lucopyranosyl (1-->4)-beta-D-fucopyranoside	*Bridelia ndellensis Mitragyna stipulosa*	82, 83
Triterpenoid saponin	*Pentaclethra macroloba*	84
Ursolic acid	*Eriobotrya japonica*	85
Quinonoid xanthene		
Ehretianone	*Ehretia buxifolia*. Roxb.	86
Resveratrol		
Resveratrol(3,4',5-trihydroxytransstilbene)	Hong Bei Si Chou	87
Alkaloid (12-methoxy-4-methylvoachalotine)	*Tabernaemontana catharinensis*	88

(*Contd.*)

Table 2—List of herbal compounds active against snake envenomation—Contd.

Compound	Plant source	Reference
Miscellaneous Chemical Groups and Compounds		
Flavonoids, quinonoid, xanthene, polyphenols and terpenoids	*Bursera simaruba, Clusia torresii, Clusia. palmana, Croton draco, Persea americana, Phoebe brenesii, Pimenta dioica, Sapindus saponaria, Smilax cuculmeca* and *Virola koschnyi*.	89
Aristolochic acid, indomethacin, quercetin, curcumin, tannic acid, and flavone		90
Acalyphin, chlorogenic acid, stigmasterol, curcumin and tectoridin	*Lonicera japonica, Hemidemus indicus, Curcuma longa*	91
SNVNF	*Strychnos nux vomica*	92
Ar- turmerone	*Curcuma longa*	93

How the herbal compounds neutralize the toxic venom constituents with in the body? Till date, there is no definite answer or mechanism. Many hypothesis have been proposed such as (1) protein precipitation hypothesis[44], (2) enzyme inactivation hypothesis[93], (3) chelation hypothesis[83], (4) adjuvant action hypothesis[52], (5) anti-oxidant hypothesis[77], (6) protein folding hypothesis, (7) combination hypothesis[52] and many more. The above hypothesis have their own limitations. Among these, protein precipitation-inactivation hypothesis is more acceptable. However, more emphasis should be focused on this area in the near future.

Future of antivenom and herbal therapy

The limitations of AVS are well known and the world is looking for an alternative for snake bite treatment. Till date no suitable alternative measures are available, except the natural herbal remedies, which are showing promising expectations. The advantages of herbal compounds are that, they are cheap, easily available, stable at room temperature and could neutralize a wide range of venom antigen. In many cases, the whole herbal extracts are more powerful than the individual herbal compounds. The herbal compounds could effectively neutralize snake venom in presence of AVS, which is another advantage of herbal compounds. It may be opined that the identified herbal compounds having AVS potentiating actions might be selected for further trials.

It is now obvious that the future of AVS is lying on the shoulder of herbal compounds and combination of these two antidotes may find a suitable alternative to the snake bite treatment in the near future. Combination therapy is an old practice of Ayurvedic medicine. Various therapeutic, ayurvedic formulations are available commercially, *eg*., Articulin-F comprising of a fixed combination of *Boswellia serrata, Withania somnifera, Curcuma longa,* Zinc for treating osteoarthritis, Trikatu comprising of black pepper, long pepper and ginger, for treating digestive ailments, etc. In ayurveda there could be combination ranging from two to twenty formulations, in fixed doses. Thus it is evident that ayurveda not only uses herbal components, but along with it various metal ions and spices are also important part of combination formulation. It is well recognized in ayurveda that a medicinal plant may need to be administered with other plants, that is in combination, in order to exert its therapeutic effect. The second plant may potentiate the action of the first, while the third might help to prevent the toxicity of the second plant. Recently emphasis has been given to the age old concept of combination therapy in several pathophysiological condition like cancer[94], tuberculosis, malaria[95] and AIDS. We are also hopeful that snake bite treatment may be benificial by this application and gradually the herbal compounds may find an alternative to the AVS.

Conclusions

Recently, the World Health Organization estimated that 80% people worldwide rely on herbal medicines for some aspect or other for their primary healthcare[96]. World Health Organization has shown great interest in documenting the use of medicinal plants used by tribals from different parts of the world. Many developing countries have intensified their efforts in documenting the ethnomedical data and scientific research on medicinal plants. Once these local ethnomedical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious herbal drug treatment and improved health status in several pathophysiological conditions including snake bite in the near future. It is our responsibility to identify, cultivate and culture these eco-friendly herbs for the alleviation of human suffering and death against snake bite.

Acknowledgement

The first author AG acknowledge the partial financial help for plant and snake venom research from ICMR, New Delhi, India and CSIR, New Delhi, India.

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