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Snake Venom Induced Local Toxicities: Plant Secondary Metabolites as an Auxiliary Therapy

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Abstract: Snakebite is a serious medical and socio-economic problem affecting the rural and agricultural laborers of tropical and sub-tropical region across the world leading to high morbidity and mortality. In most of the snakebite incidences, victims usually end up with permanent tissue damage and sequelae with high socioeconomic and psychological impacts. Although, mortality has been reduced markedly due to anti-venom regimen, it is associated with several limitations. Snake venom metalloprotease, hyaluronidase and myotoxic phospholipase A_2 are the kingpins of tissue necrosis and extracellular matrix degradation. Thus, inhibition of these enzymes is considered to be the rate limiting step in the management of snakebite. Unfortunately, tissue necrosis and extracellular matrix degradation persists even after the administration of anti-venom. At present, inhibitors from snake serum and plasma, several synthetic compounds and their analogs have been demonstrated to possess anti-snake venom activities, but the use of plant metabolites for this purpose has an added advantage of traditional knowledge and will make the treatment cheaper and more accessible to the affected population. Therefore, the clinical and research forums are highly oriented towards plant metabolites and interestingly, certain phytochemicals are implicated as the antibody elicitors against venom toxicity that can be exploited in designing effective anti-venoms. Based on these facts, we have made an effort to enlist plant based secondary metabolites with anti-ophidian abilities and their mechanism of action against locally acting enzymes/toxins in particular. The review also describes their functional groups responsible for therapeutic beneficial and certainly oblige in designing potent inhibitors against venom toxins.

Keywords: Hyaluronidase, Local toxicity, Phytochemicals, PLA_2, Snake venom, SVMPs, Synthetic drugs.

INTRODUCTION

Snake envenomation is a serious medical and socio-economic crisis affecting the healthy individuals like children, rural population, specially the agricultural laborers in tropical and sub-tropical regions. Snakebite has become a nightmare for the inhabitants of southern Asia, northern Africa, Latin America and the Middle East nations. The affected regions experience high mortality and morbidity rates because of poor access to health services. In addition, most of the snakebite victims end up with permanent tissue damage and sequelae, with high socioeconomic and psychological impacts. Although the accurate statistics of snakebite incidence is difficult to estimate, available data reported of about 5 million cases of snakebites globally per annum, of which 3.75 million people severely get affected affected and results in 1.25 million deaths [1, 2]. About 2,50,000 snake bite incidences are recorded in India every year and resulting in more than a 100,000 deaths and most of them go unnoticed [3, 4]. In view of this, snakebites are being considered and recognized as a major public health hazard across the world and may therefore be appropriately categorized as a neglected tropical disease by world health organization [1].

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Of the 3000 known species of snakes 410 are venomous and are particular to tropical and subtropical regions of the world. There are about 53 venomous snakes in India of which majority of the bites and mortality are attributed to species like *Naja naja* (spectacled cobra), *Daboia russelli* (Russell's viper), *Bungarus caeruleus* (common krait), *Echis carinatus* (saw-scaled viper), and *Ophiophagus hannah* (king cobra). The composition of venom varies with the species and the variation was observed due to the differences in age, sex, habitat, diet and season. Hence, the individual venoms would exert varied spectrum of pharmacological properties [5, 6].

Snake venom is an awfully composite concoction of pharmacologically active enzymatic and non-enzymatic protein and peptide toxins that assist in prey immobilization mainly as hypotensive, paralytic and digestive aids. There are more than twenty different enzymes present in venom including phospholipases, snake venom metalloproteases (SVMPs), snake venom hyaluronidases (SVHYs), serine proteases, phosphatases (acid and alkaline), acetyl cholinesterase, transaminase, phosphodiesterase, nucleotidase, ATPase and nucleosidases [7].

Snake envenomation occurs due to subcutaneous/ intramuscular injection of venom into the human victims resulting in complicated pharmacological effects that depend on the combined and synergistic action of toxic and non-toxic

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components. The pathophysiology of snake envenomation includes both local and systemic effects [8]. The magnitude of toxicity depends on the dose and potency of the toxins and their diffusion rate in to the general circulation from the site of bite. However, the extent of systemic toxicity greatly varies depending on the body mass for an identical bite. Therefore, envenomation may include only local effects (hemorrhage, edema, myonecrosis, and extracellular matrix (ECM) degradation) or may include systemic effects (neurotoxicity, myotoxicity, cardiotoxicity, and alterations in hematological systems) [7, 9]. Based on the major constituents of venom and associated pharmacological effects, snakes are also classified as neurotoxic (cobras and kraits), hemorrhagic (vipers) and myotoxic (sea snakes). It is now well known that such a strict categorization is not valid as each species may result in any kind of manifestation [2].

The mortality rate due to snakebite has reduced markedly by the use of anti-venoms, the only medically approved remedy for snakebites [10]. At present 20 different nations are involved in the production of therapeutic anti-venoms (http://www.toxinfo.org/anti-venoms/Index_Product.html). The monovalent (prepared against single species of snake venoms) and polyvalent (prepared against mixture of selected species of snake venoms) anti-venoms against 55 clinically important snake venoms are available. In addition, anti-venoms are also available against specific snake species [2]. The commercially available anti-venoms are highly efficient in neutralizing the systemic toxicity induced by the target specific toxins. However, the anti-venom therapy is associated with various limitations such as serum sickness, anaphylactic reactions, unavailability, lack of information on the bitten snake species and more importantly it offers no protection against local tissue damage. Due to the limitations of anti-venom therapy, researchers are in continuous search of new molecules with potential application as alternative therapeutics that complements the existing anti-venom therapy in order to neutralize mainly the local tissue damages. In this regard, the plant extracts and their purified compounds and derivatives occupy the lead position followed by anti-venom components from the snake sera, and synthetic compounds.

Several articles and reviews have been dedicated to preparation, efficacy determination and safety evaluation of anti-venoms [11, 12]. There are many reports showing the presence of inhibitors in the sera and plasma of the snakes (venomous and non-venomous), which can neutralize the toxins present in their own venom and as well as in venom toxins of different species [13-15]. In addition, synthetic molecules and their analogs have also been demonstrated to possess anti-snake venom activities and could be better and efficient agents for ameliorating both local and systemic effects to complement the existing therapies [2, 16-18]. However, only few attempts have been made to understand the possible reasons for venom induced persistent tissue necrosis. Therefore, in the present review, an attempt has been made to provide information about the agents responsible for tissue damage and their neutralization by secondary metabolites of medicinal plants. The review also illustrates an overview of the field, discussing the available data on the plant therapeutic agents with venom neutralizing abilities and their mechanism of action describing the functional groups of phytochemicals responsible for their therapeutic beneficial against locally acting enzymes/toxins in particular.

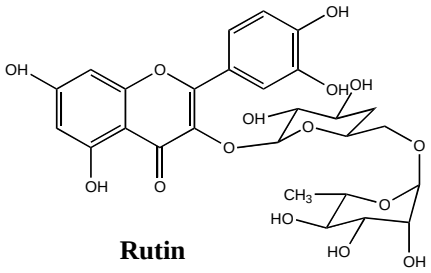
PLANT METABOLITES: AN AUXILIARY THERAPY IN THE TREATMENT OF SNAKE ENVENOMATION

Medicinal plants constitute rich source of therapeutically active molecules and have proven to be very effective and safe in the treatment of several pathophysiological disorders like inflammation, wound healing, arthritis, diabetes, liver and kidney associated problems [19-23]. Further, plant metabolites and their structural analogs with anti-venom activity (Fig. **1**) could be used directly in the treatment of ophidian accidents or indirectly as a supplement to conventional anti-venin therapy [24-26]. The use of plant extracts/metabolites with anti-ophidian properties, as an antidote to treat life-threatening snakebite complications is an ancient option for many tribes and communities till today, which do not have access to anti-venin therapy in the tropical and subtropical regions of the world. Plant extracts/isolated compounds are reported to inhibit edema, inflammation, hemorrhage, myotoxicity and neurotoxicity induced by venom. [2, 4, 24-27]. Topical application of the plant based material onto the bitten area, chewing leaves/seeds or barks or drinking plant extracts or decoctions or injecting the extracts are some procedures intended to counteract snake envenomation. However, most of them lack scientific validation, as experimentally validated phytochemicals will certainly be beneficial for the development of more effective drugs. Besides, the use of plant metabolites for this purpose will make the treatment cheaper and more accessible to the affected population living in resource poor conditions and away from the primary health centers. At present, attempts are being made to neutralize venom toxicity by isolated/purified compounds from medicinal plants.

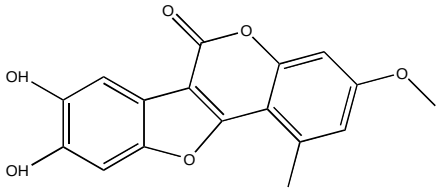
SNAKE VENOM INDUCED LOCAL MANIFESTATIONS

The earliest manifestation of snakebite is the local toxicity and features are noted within 5-8 mins. Envenomations inflicted by *viperid* and *crotalid* are characterized by prominent local tissue damage, which develops rapidly after the bite and often results in permanent sequelae [28, 29]. The consequences include local pain with radiation; edema, hemorrhage, myonecrosis and tissue damage following bleeding and tissue softening at the bitten site [30, 31]. Edema is considered to be the multifactorial pathological condition that results due to the synthesis of potent autocoids or eicosanoids, and damage in the microvasculature with plasma extravasations, and the release of cytokines, nitric oxide, kinins, and histamines are triggered by venom components [32].

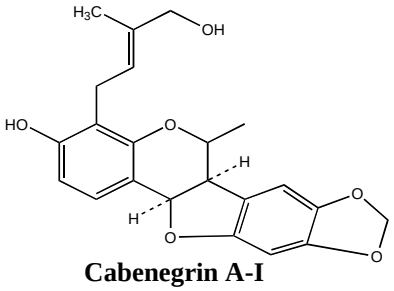
Hemorrhage and myonecrosis occur as a result of the combination of several factors present in venom. In addition to myotoxic PLA_2s, degradative action of SVMPs and SVHYs activities are also found to increase vascular permeability by softening the tissue and generating pro-inflammatory end products. These hemorrhagins, together with secondary factors can produce incoagulability of blood



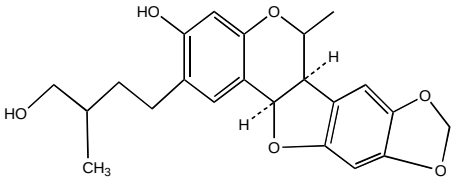
Rutin



Wedelolactone



Cabenegrin A-I



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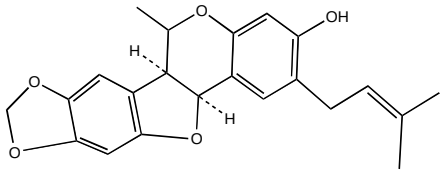
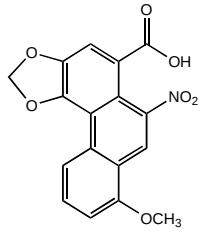
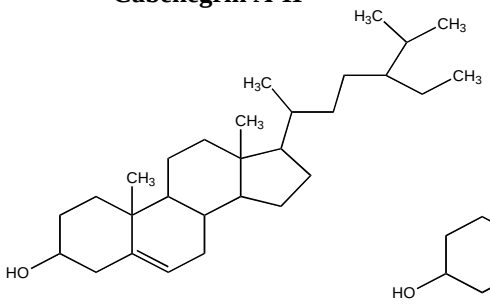


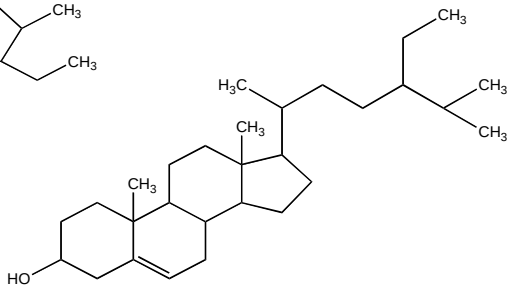
Edunol



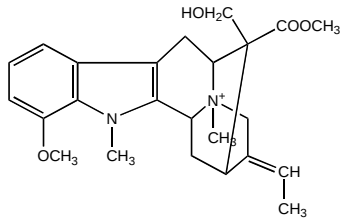
Aristolochic acid



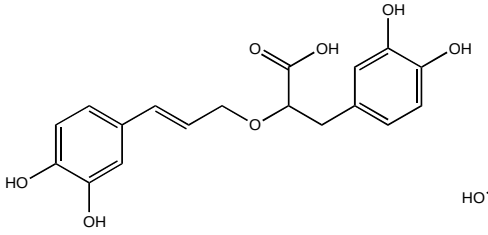
Sistosterol



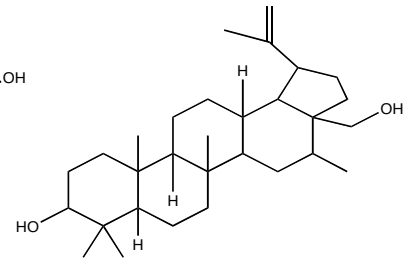
Stigmasterol



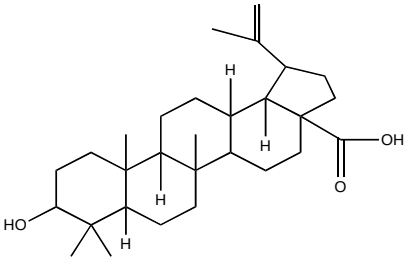
MMV



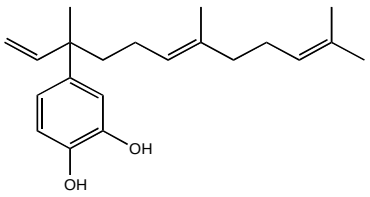
Rosmarinic acid



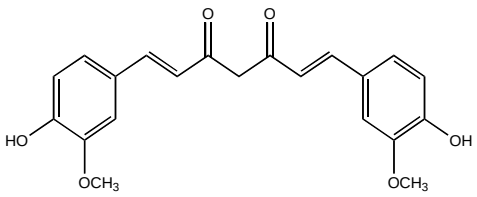
Betulin



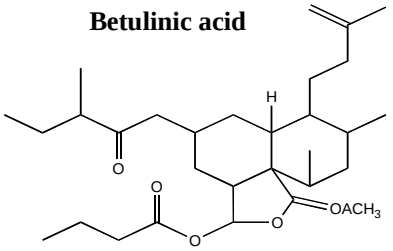
Betulinic acid



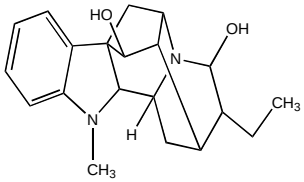
4-nerolidyl catechol



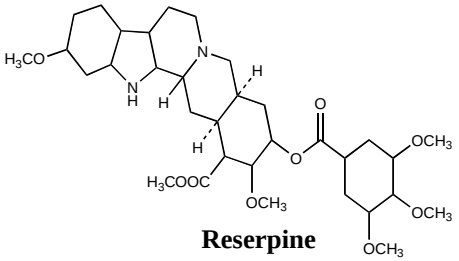
Curcumin



Clerodane diterpenoid

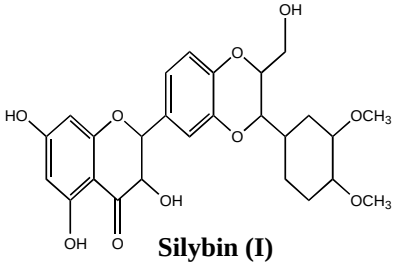


Ajmaline



Reserpine

(a1)



Silybin (I)

(Fig. 1) contd….

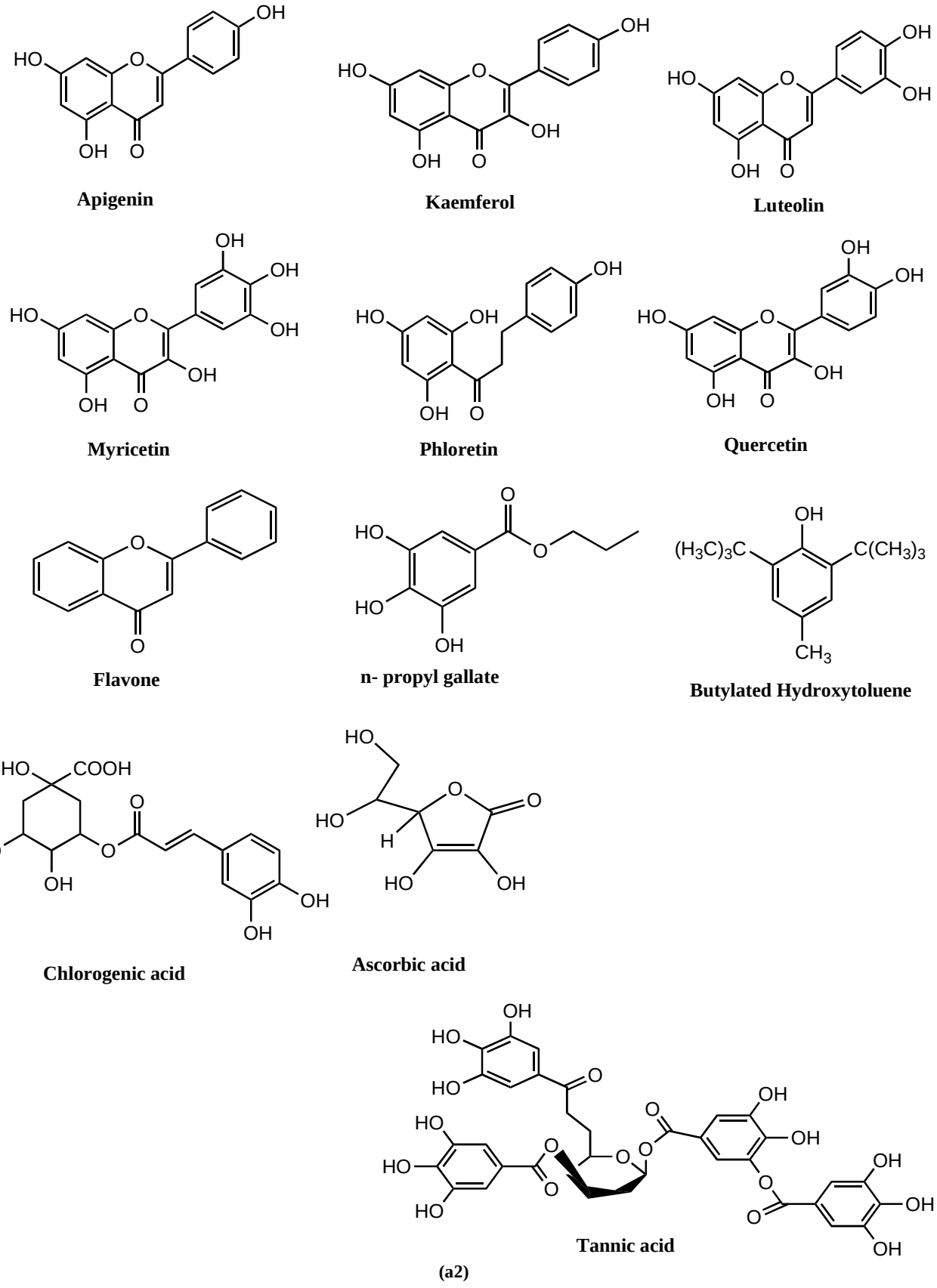


Fig. (1). Structure of anti-ophidian phytochemicals isolated from medicinal plants

allowing not only an altered permeability of the blood vessels, but also inducing the exit of plasma and red blood cells to extra-vascular spaces. Further, thrombogenic enzymes present in viper venom promote the formation of weak fibrin clot, which in turn activates the plasmin and results in a consumptive coagulopathy and hemorrhage [33, 34]. Myonecrosis may be due to the vascular degeneration and ischemia caused by SVMPs or due to the direct action of myotoxins (enzymatic/non-enzymatic) on the plasma membrane of muscle cells, which results in extensive muscle tissue damage which in turn results in painful and restricted muscle movement [35 - 38]. Furthermore, the end products generated by ECM degrading enzymes are pro-inflammatory in nature and tend to induce target cells at the bitten region to secrete inflammatory mediators [39, 40]. Therefore, it can be concluded that inhibition of these factors is the rate-limiting step in the course of snakebite management. Inhibition of SVMPs, myotoxic PLA_2 and SVHYs is not only minimizes the brutal local tissue damage but also slow down the diffusion of target specific toxins thereby increasing the survival time of the victims [41-44]. Furthermore, inhibition of these factors blocks the persistent tissue necrosis that continues even after the administration of antivenins.

SNAKE VENOM PHOSPHOLIPASE A_2: PLANT METABOLITES AS INHIBITORS

Phospholipase A_2 (PLA_2, EC 3.1.1.4), are a group of enzymatic toxins abundantly present in snake venoms. These enzymes catalyze the hydrolysis of the sn-2 acyl bond of glycerophospholipids, in a calcium- dependent fashion generating free fatty acids and lysophospholipids. Snake venom PLA_2s are considered to be target specific and mimic the whole venom poisoning by exhibiting a wide variety of toxic effects. Venom PLA_2s belong to class I (terrestrial elapids and sea snake venoms), II (viperid snake venoms) and III (bee and lizard venoms), among the growing family of secreted PLA_2s [45, 46]. According to the recent phylogenetic study, snake venom PLA_2s can be classified into two groups based on their evolutionary derivation, i) the calcium-dependent catalytically active enzymes (Asp49-, Asn49- and Gln49-PLA_2s) and ii) the catalytically inactive PLA_2s that exert their effects through a calcium-independent mechanism (Lys49-, Arg49- and some Asp49-PLA_2s).

Furthermore, type II PLA_2s are the chief toxins responsible for the expression of inflammatory, vasodilating and vasoconstriction mediators including prostaglandins, histamine, kinins, eicosanoids, platelet activating factor, catecholamines, dopamine, nitric oxide and endothelins during envenomation [47, 48]. The PLA_2s are also responsible for the hydrolysis of membrane phospholipids that release arachidonic acid, which serves as substrate for pro-inflammatory or pro-coagulant mediators such as prostaglandins, leucotriens, thromboxanes and platelet activating factor leading to systemic toxicity. In addition, PLA_2 are also involved in exerting the local effects including edema and myonecrosis. Myotoxic PLA_2s are basic and belong to the Lys49 family of catalytically inactive PLA_2s, which cause irreversible damage to skeletal muscle fibers through a yet poorly understood mechanism. These myotoxic PLA_2s are devoid of catalytic activity due to the substitution of key residues corresponding to amino acids 95-117 which includes the myotoxic site of Lys49-PLA_2s at the calcium-binding loop in the C-terminal region [37, 49-52].

On the other hand, during snakebite treatment regime, administered monovalent/polyvalent anti-venin can neutralize the systemic toxicities induced by target specific PLA_2 but provide no protection against myonecrosis induced by myotoxins (enzymatic/non-enzymatic). Therefore, inhibition of myotoxins is the rate-limiting step in the snakebite management. Many studies have shown that the binding of the substrate to PLA_2 occurs through a well-formed hydrophobic channel. Therefore, blocking the hydrophobic channel is an effective way to inhibit PLA_2. Inhibitors of PLA_2 derived from medicinal plants are of different chemical forms like glycoproteins, lanostanoids, phenylpropanoids, alkaloids, sterols, antioxidants, polyphenols, flavonoids, terpenoids and glucosides are mentioned (Table **1**).

Machaiah and Gowda, [53] isolated a glycoprotein inhibitor (WSG) from *Withania somnifera* with a molecular mass of 27 kDa that could inhibit the toxic PLA_2s from cobra venom. The authors suggest that neutralization of the pharmacological effects is due to the formation of a complex between WSG and the toxic PLA_2s thereby inhibiting the enzyme activity. WSG is an acidic glycoprotein similar to the α-chain of the snake plasma phospholipase inhibitors (PLIs) but in contrast it is composed of a single subunit [13].

Further, Chethankumar and Srinivas [54] have isolated turmerin, a protein from *Curcuma longa L.* with a relative molecular mass of 14 kDa. The protein turmerin, rich in proline, inhibits the PLA_2 activity of *Naja naja* venom (NV) thereby neutralizing the pharmacological properties, at a 1:2.5 molar ratio of NV-PLA_2: turmerin. A Lineweaver-Burk plot indicated that turmerin follows a linear mixed type of inhibition.

Lindahl and Tagesson [55] analyzed the inhibitory effect of flavonoid rutin on group I PLA_2 from *Naja naja* and group II PLA_2s from *Vipera russelli* and *Crotalus atrox.* Rutin efficiently inhibited group II PLA_2 but the inhibition was to a lesser extent for group I PLA_2. Results indicated the importance of hydroxyl group in position 5, as well as the double bond and the double bonded oxygen in the oxane ring for the overall ability of flavonoids to exert PLA_2 inhibition, and that the hydroxyl groups in positions 3 and 4 are required for selective inhibition of group II PLA_2s.

Melo and Ownby [56] demonstrated inhibitory efficacy of Wedelolactone, a coumestan from the plant *Eclipta prostrata* against different myotoxic PLA_2s. Wedelolactone was found to be active against South American crotalid venoms such as *C. d. terrificus*, *B. jararaca*, *B. jararacussu* and *Lachesis muta*, as well as the North American crotalids *C. viridis viridis* and *Agkistrodon contortrix* [24, 57]. In addition, wedelolactone analogs also inhibited the myotoxicity induced by *B. jararacussu* venom by antagonizing the creatine kinase (CK) release in skeletal muscle [58].

Cabenegrin A-I and A-II are the two pterocarpans isolated from the plant *cabeça-de-negro* (negro´s head), the main ingredient in *Específico Pessoa*, a Northeast Brazilian folk medicine used against snakebites. Pterocarpans prenylated

Table 1. List of Plant Metabolites Effective against Snake Venom PLA_2

Chemical Form	Name of the Compound	Name of the Plant Species	Snake Genera	References
Protein	Glycoprotein (WSG)	*Withania somnifera*	*Naja naja*; *Daboia russelii*	[53]
	Tumerin	*Curcuma longa L*	*Naja naja*	[54]
Flavonoid	Tectoridin	*Pueraria thunbergiana*	*Naja naja*	[69]
	Rutin; Curcumin	*Asperagus, Rheum* *Curcuma longa*	*Vipera russelli*; *Crotalus atrox*	[55, 69]
Pterocarpans	Cabenegrins (A-I & A-II)	*Cabeça-de-negro*	Vipers	[59]
	Edunol	*Harpalyce brasiliana*		[60]
Alkaloid	Aristolochic acid	*Aristolochia radix*	*Vipera russelli*	[61]
	12-methoxy-4-methylvoachalotine	*Tabernamontana catharinensis*	*Crotalus durissus terrificus*	[65]
	Berberine	*Cardiospermum halicacabum*	*Vipera russelli*	[70]
Sterols	Sitosterol; Stigmasterol	*Eclipta prostrata*	*C. d. terrificus*	[65,69]
Coumestan	Wedelolactone,	*Eclipta prostrata*	*C. d. terrificus*; *B.jararaca*, *B. jararacussu*	[56, 65]
polyphenols	Rosmarinic acid	*Cordia verbenacea*	*Bothrops pirajai*	[63]
	Chlorogenic acid		*Vipera russelli*	[69]
Terpenoids/ Saponin	Clerodane; Betulin; Betulinic acid; 4-nerolidylcatechol;	*Baccharis trimera*; *Betula alba* *Piper* sp	*B. neuwiedi*; *B. jararacussu*; *Bothrops* sp; Vipers; *Naja nigricolis*	[66,67]
Glucosides	Acalyphin	*Acalypha indica*	*Vipera russelli*	[69]
Others	AIPLAI	*Azadirachta indica*	*Vipera russelli*; *Naja naja*; *Naja kaouthia*	[68]

in the A-ring are described as very potent compounds [59]. In addition, a new pterocarpan denominated as edunol was isolated from the root of *Harpalyce brasiliana* and demonstrated to possess anti-myotoxic, anti-proteolytic and anti-PLA_2 properties [60].

The alkaloid aristolochic acid (8-methoxy-6-nitrophenanthro (3,4-d-1,3-dioxole-5-carboxylic acid) isolated from *Aristolochia radix* inhibits enzymatic and pharmacological activities of a basic PLA_2 from *Vipera russelli* venom. The alkaloid inhibits the edema-inducing activity as soon as it reaches the site, but does not aid in recovery. The interaction of aristolochic acid with PLA_2 was followed by circular dichroism measurements. Binding of aristolochic acid causes a change in the secondary structure of the PLA_2 that is characterized by an increase in the apparent content of alpha-helix, without any detectable change in the tertiary structure of PLA_2. Further, Aristolochic acid showed a dose-dependent inhibition of phospholipid hydrolysis by group-IIA human PLA_2, porcine pancreatic PLA_2, and snake venom (*Naja naja*) PLA_2. The sensitivity of these PLA_2s to inhibition by aristolochic acid varied markedly: group IIA PLA_2 > *N. naja* PLA_2 > porcine pancreatic PLA_2 [61]. The crystal structure of a complex formed between *Vipera russelli* PLA_2 and aristolochic acid has been determined and it was found that one molecule of aristolochic acid specifically binds to one of the two crystallographically independent molecules of PLA_2 in the form of an asymmetric dimer [62].

Further, Ticli *et al.*, [63] reported the anti-inflammatory and antimyotoxic properties of rosmarinic acid (RA) against *Bothrops jararacussu* snake venom and isolated PLA_2s. RA inhibited the edema and myotoxicity induced by basic PLA_2s such as BthTX-I and BthTX-II. The authors have also demonstrated the possible mechanism of inhibition of an isolated PLA_2 (Lys49-PLA_2 BthTX-I) by RA using a molecular model of the complex. The RA was modeled into the hydrophobic channel leading to the active site. After

energy minimization, the RA remained in the hydrophobic channel with aromatic rings. The hydroxyl group of one of the aromatic rings bound to His48 and the carboxyl group bound to the Lys69 (Fig. **2**). This could be the possible reason for PLA_2 inhibition by RA-PLA_2 interaction. Generally, the His48 of the catalytic network is firmly conserved in class II PLA_2s. The majority of PLA_2-inhibitor complexes have functional side group bound to His48 [52].

In addition, clerodane, a diterpenoid (Bt-CD) from *Baccharis trimera* was proven to be an anti-ophidian compound by exhibiting anti-proteolytic and anti-hemorrhagic properties against snake venoms. Further, Bt-CD partially inhibited the edema induced by PLA_2s (basic and acidic), crude venom, and metalloproteases [64]. Further, wedelolactone and sitosterol from the plant *Eclipta prostrata* (*Asteraceae*) and stigmasterol and 12-methoxy-4-methylvoachalotine (MMV) from *Tabernaemontana catharinensis*, were shown to inhibit the myotoxic PLA_2 activity and lethality of the *Crotalus durissus terrificus* venom [24, 65].

The pentacyclic triterpenes such as betulin and betulinic acid from *Betula alba* showed PLA_2 inhibition with greater potency. The result confirmed the importance of a carboxylate group for the exertion of PLA_2 inhibition. Docking studies predict that betulinic acid can be inserted into the PLA_2 binding site with correct energy values [66]. Furthermore, another terpenoid compound, 4-nerolidylcatechol from *Piper* species exhibited inhibition towards PLA_2 and myotoxic effects of purified myotoxins from *Bothrops* species venoms [67].

Mukherjee *et al.*, [68] isolated a compound AIPLAI (*Azadirachta indica* PLA_2 inhibitor) from the leaf of *A. indica*, which inhibited PLA_2 from the cobra and *Russell's viper* venom in a dose-dependent manner. Kinetic study revealed that the AIPLAI inhibited the *N. kaouthia* PLA_2 in a non-competitive manner. Further, Nirmal *et al.*, [69] studied the structural relationship between medicinally important herbal compounds such as acalyphin, chlorogenic acid, stigmasterol, curcumin and tectoridin and PLA_2 from Russell's viper. The molecular modeling studies revealed favorable interactions with the amino acid residues at the active site of venom PLA_2 that could result in the inhibition. Chandra *et al.*, [70] have analyzed the inhibition of PLA_2 from Russell's viper venom using isoquinoline alkaloid, berberine from *Cardiospermum halicacabum*. According to the X-ray crystallographic studies, berberin was found to form complex with PLA_2 by positioning itself in the active site of the enzyme. Berberin, being hydrophobic made a number of hydrophobic contacts with side chains of neighboring amino acids in addition to hydrogen bonds with Gly 30 and His 48 of the enzyme. According to the inhibition studies berberine was found to be the competitive inhibitor of PLA_2.

Recently, Dos Santos *et al.*, [71] proposed an x-ray crystallographic model to study the interaction of RA with

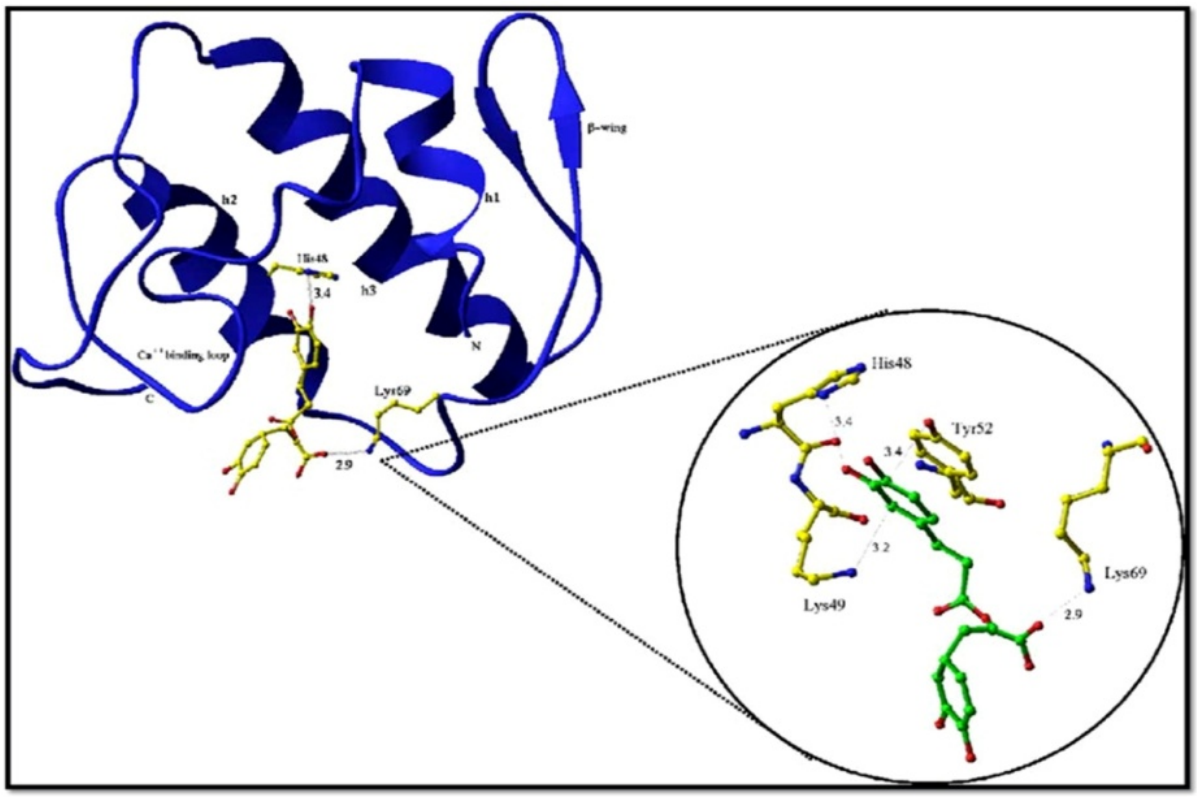


Fig. (2). Molecular model of rosmarinic acid and monomeric BthTX-I complex. Drawn with the program RIBBONS. The residues interacting with the rosmarinic acid and the BthTX-I are shown in ball-stick representation. (The figure has been adopted with permission from Elsevier, Ref; Ticli *et al.*, 2005).

PrTX-I, a Lys49-PLA_2 from *Bothops pirajai* snake venom. The crystal structure PrTX-I- RA complex showed that the inhibitor interacts with the toxin at the entrance of its hydrophobic channel (Fig. **3**), demonstrating interaction of the ligand at the well characterized "myotoxic site" at the C-terminus of the Lys49-PLA_2 thereby affecting toxin's ability to destabilize the muscle membranes. According to the authors the inhibitory effect of RA is due to the steric hindrance that blocks the access of substrates to the hydrophobic channel. This hypothesis was further supported by comparison of the PrTX-I/RA complex with structures of two Lys49-PLA_2s bound to fatty acids (PDB ID 1QLL and 1XXS) suggests that RA impairs the binding of lipid tails to the hydrophobic channel by physically blocking its entrance [52]. Altogether, the PLA_2 inhibition by plant derived secondary metabolites could be due to direct binding to the enzyme or chelating the divalent metal ion Ca^{2+}, the cofactor for PLA_2. Some phytochemicals by denaturating or by binding to the specific domains or by favorable interactions with the amino acid residues involved in the catalysis exert their inhibition towards PLA_2.

SVMPs AND THEIR NEUTRALIZATION BY PLANT THERAPEUTICS

SVMPs are zinc-dependent enzymes of varying molecular mass, abundant in viperid and colubrid venoms, and to a lesser extent in elapid venoms. They belong to a reprolysin subfamily of the metalloproteinases containing a conserved zinc-chelating sequence, HEXXHXXGXXH….M. Based on their molecular size and presence of other domains; SVMPs are categorized into four groups, P-I to P-IV [72-74]. The P-I group of SVMPs are low molecular mass, non-glycosylated enzymes, composed only of the catalytic domain with the characteristic zinc-binding site followed by a methionine turn. The zinc atom is tetrahedrally co-ordinated by three histidine residues and a water molecule. The P-II SVMPs are disintegrin precursors with an additional disintegrin domain carboxy to the catalytic domain. The P-III SVMPs are high molecular mass proteinases that contain both disintegrin-like and cysteine rich domain carboxy to the proteinase domain. Both P-II and P-III SVMPs are glycosylated, high molecular mass enzymes and significant reduction in their hemorrhagic activity with deglycosylation suggests the role of carbohydrate moieties. The P-IV SVMPs are high molecular mass proteinases with similar domain structure to that of P-III class with an additional C-type lectin domain [34, 72, 74-76].

The specificity of action varies among different SVMPs. These proteases degrade a variety of protein molecules present in ECM of basement membrane, membrane proteins, integrins, immunoglobulins, tissues surrounding blood vessels and capillaries, and as well as plasma proteins resulting in disorganization of cell-matrix and cell-cell adhesion processes

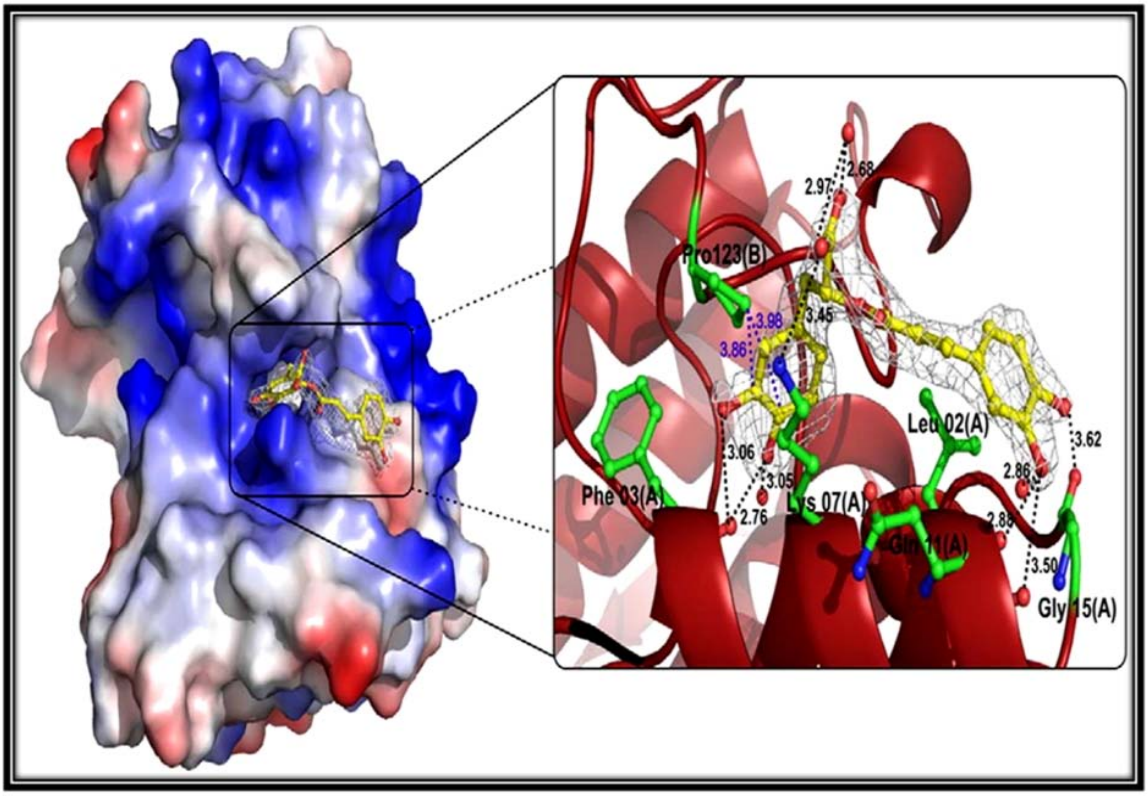


Fig. (3). Interactions between RA and PrTX-I atoms in the PrTX-I/RA complex. The surface charge distribution for the PrTX-I/RA crystallographic model and specific interactions between RA with some PrTX-I atoms are shown. Only the interactions with interatomic distances shorten than 3.7 A° are represented between chain A and the RA molecule (black dashes). To represent the interactions between RA and the residue Pro123 of chain B a larger distance cut-off was considered (blue dashes). Residues whose contacts with RA are established through water molecules are not shown. (The figure has been adopted with permission from PLOSOne, Ref; dos Santos *et al.*, 2011).

[77]. In addition, SVMPs also alter the hemostasis by targeting proteins and cells of the blood coagulation process such as clotting factors, fibrinogen, von willebrand factor and platelets, and also affect cytokine expression profile [78-81]. Several studies have demonstrated the indirect effects of SVMPs, including alterations in the expression of pro-inflammatory and pro-apoptotic genes of both human fibroblasts and umbilical vein endothelial cells in which up-regulation of pro-inflammatory genes was observed [34, 82-83].

SVMPs induce local effects including hemorrhage, myonecrosis, edema, blistering, dermonecrosis and inflammation in addition to systemic toxicity including systemic hemorrhage, coagulation, defibrinogenation and alteration in platelet aggregation [33, 81, 84]. Therefore, inhibitors of SVMPs not only reduce the local effects but also prevent the secondary complications of snakebite by ameliorating the systemic hemorrhage. Several studies reported that plant metabolites as SVMP inhibitors are of different chemical forms namely terpenoids, saponins, sterols, alkaloids, antioxidants, polyphenols, flavonoids, pthalates and other compounds (Table **2**).

The triterpenoid saponins isolated from the plant *Pentaclethra macroloba* namely macrolobin A & B that inhibited the hemorrhagic activity of *Bothrops* venoms and isolated *B. neuwiedi* and *B. jararacussu* venoms. Further, the saponins dose dependently inhibited the fibrin(ogen)olytic activity induced by metalloprotease enzymes. Proteolytic activity induced by isolated class P I and P III metalloproteases was inhibited by macrolobin A up to ~90% and 80% respectively at a ratio of 1:15 (protease: inhibitor; w/w) [85].

A clerodane diterpenoid, (CD) isolated from the Brazilian medicinal plant *Baccharis trimera* (Less) (Bt), reported to possess anti-proteolytic and anti-hemorrhagic properties against snake venoms. Bt-CD exhibited complete inhibition of hemorrhage and proteolytic activity caused by Bothrops snake venoms. The inhibitor neutralized the hemorrhagic, fibrinogenolytic and caseinolytic activities of class P-I and III metalloproteases isolated from *B. neuwiedi* and *B. jararacussu* venoms. In addition, Bt-CD partially inhibited metalloproteases induced edema [64].

Table 2. List of Isolated Plant Metabolites Effective against SVMPs

Chemical Form	Name of the Compound	Plant Source	Snake Genera	References
Terpenoids/ saponin	Macrolobin A & B	*Pentacletra macroloba*	*B. neuwiedi*; *B. jararacussu*	[85]
	Lupeol acetate	*Hemidesmus indicus*	*Daboia russelli*; *Naja kaouthia*	[87]
	Neo-clerodane	*Baccharis trimera*	*B. neuwiedi*; *B. jararacussu*	[64]
Sterols	β-sitosterol; Stigmaterol Wedelolactone;	*Pluchea indica (Less)*	Cobra, Viper	[86, 57]
		Eclipta prostrata	*B. jararaca*; *B. jararacussu*; *Lachesis muta*; *Calloselasma rhodostoma*	[89,57]
Acids	2-hydroxy-4-methoxy benzoic acid	*Hemidesmus indicus*	*Vipera russelli*	[88]
	Anisic acid	*Pimpinella anisum,*		
	Rosmarinic acid	*Argusia argentea*	*T. flavoviridis*; *Crotalus atrox*; *Gloydius blomhoffi*; *Bitis arietans*	[91]
Phenols	Pentagalloyl; Glucopyranose; Methyl gallate; Gallic acid	*Mangifera indica*	*Calloselasma rhodostoma;* *Naja naja kaouthia*; *Vipera russelli*	[90]
Flavonoids	Isoquercitrin; Myricetin-3-O-glucoside; Catechin; Gallocatechin	*Schizolobium parahyba*	*B. jararacussu*; *B. neuwiedi*	[94]
Pthalate	Di- iso-butyl phthalate	*Emblica officinalis*	*Vipera russelli*; *Naja naja*	[93]
Others	SNVNF	*Strychnosnux vomica*	*Daboia russelli*	[92]

Gomes *et al.*, [86] reported the isolation of β-sitosterol and stigmasterol from the methanolic root extract of the Indian medicinal plant *Pluchea indica* (Less). The active fraction was found to neutralize the viper venom-induced lethal, hemorrhagic, defibrinogenation and edema. In addition, *N. naja* venom-induced lethality, cardiotoxicity, neurotoxicity, and respiratory changes were also antagonized by the active component in addition to ameliorating the venom-induced changes in lipid peroxidation and superoxide dismutase (SOD) activity. The isolated compounds also potentiated commercial snake venom antiserum action against venom-induced lethality in experimental animals suggesting that the β-sitosterol and stigmasterol may play an important role along with antiserum, in neutralizing snake venom-induced pharmacological effects.

Chatterjee *et al.*, [87] reported the isolation of lupeol acetate from the methanolic root extract of the *Hemidesmus indicus* (L.). Lupeol acetate significantly neutralizes the lethality, hemorrhage, defibrinogenation and edema induced by *Daboia russelli* venom. Further, *N. kaouthia* venom induced lethality, cardiotoxicity, neurotoxicity and respiratory changes were also neutralized in experimental animals. Lupeol acetate enhanced the protection by antiserum action against *Daboia russelli* venom induced lethality in male albino mice. In addition, lupeol acetate antagonized venom-induced changes in lipid peroxidation and SOD activity. Further, 2-Hydroxy-4 Methoxy-Benzoic Acid isolated from the root of *Hemidesmus indicus*, efficiently neutralized viper venom induced lethality, inflammation, hemorrhage, fibrinolytic activity and defibrinogenation. The compound in addition to neutralizing the viper venom-induced actions also reduced venom induced free radical generation & showed antiserum action potentiation [88].

The anti-hemorrhagic and anti-myotoxic effects of three isolated compounds wedelolactone (WE), stigmasterol (ST) and sitosterol (SI) from *Eclipta prostrata* (EP) were investigated [57]. The myotoxicity of crotalid venoms (*Bothrops jararacussu*, *B. jararaca*, and *Lachesis muta*), purified myotoxins (bothropstoxin, BthTX, bothropasin, and crotoxin), and polylysine was quantified *in vitro* by the release rate of creatine kinase (CK) from rat or mouse extensor digitorum muscles, and *in vivo* by the plasma CK activity in mice. WE effectively neutralized the *in vitro* myotoxicity of the *crotalid* venoms and myotoxins than ST and SI. The *in vivo* myotoxic activity of venoms and myotoxins was neutralized by pre-incubation with the EP extract or WE. Intravenous administration of the WE attenuated the increase in plasma CK activity induced by subsequent intramuscular injections of the *crotalid* venoms or the myotoxins. EP and WE inhibited the proteolytic and hemorrhagic activities of *B. jararaca* venom.

Pithayanukul *et al*., [89] reported the anti-venom potential of *Eclipta prostrata* against *Calloselasma rhodostoma* Kuhl (Malayan pit viper, MPV) venom. The partially purified ethyl acetate extract (PEE) was found to contain 47% wedelolactone as its major constituent. PEE and wedelolactone dose-dependently demonstrated strong anti-proteolytic and anti-hemorrhagic activities against MPV venom. Wedelolactone at 5 mg/mL concentration could neutralize the proteolytic activity at around 76% and, at doses of 0.25-1 mg/mL, offered protection against hemorrhagic activity of the venom in the range 3–35%. Further, Pithayanukul *et al.*, [90] studied the inhibitory effects of pentagalloylglucopyranose (PGG), Methyl gallate (MG) and Gallic acid (GA), the major phenolic principles from the seed kernels of Thai mango against the proteolytic activities of *Calloselasma rhodostoma* (CR) and *Naja naja kaouthia* (NK) venoms. The authors docked the structure of rhodostoxin (P-I SVMP) from CR venom and kaouthiagin (P-III SVMP) from NK venom with GA, MG and PGG.

The molecular docking studies revealed the possible molecular orientation of the constituents (GA, MG and PGG) in the rhodostoxin-binding pocket of CR venom (Fig. **4A**). The docked conformations revealed the strong H-bond with an oxygen atom of Glu143 side chain and another interaction with other residues in the active site. From the docked conformation of GA, H-bond interactions were also found with Lys110, Ala111, Tyr112, His146, and Arg151 (Fig. **4B**). The closest distance between Zn^{2+} atom and GA's atom was 3.89 Å, which was the distance between Zn^{2+} atom and O9 atom of GA. In case of MG, the O9 atom of docked conformation showed an interaction with the Zn^{2+} atom with a distance of 2.41 Å. The other interactions were the H-bond interactions formed with Asn106, Ile108, Gly109, His152, and Ser169 (Fig. **4C**). According to the (Fig. **4D**), authors concluded that the docked PGG conformation revealed a similar interaction between both docked GA and MG conformations. Further, H-bond interactions were also found with Asn106, Ile108, Gly109, Lys110, Ala111, Tyr112, Arg151, His152, and Ser169. Moreover, H-bond interactions were found with Lys105, Ile107, Leu113, Asp114, His142, Val150, His170, and Ile171. The interaction between the Zn^{2+} atom and the O29 atom of docked PGG conformation was shown to have a distance of 2.49 Å [90].

Further, the molecular docking study revealed that PGG was bound firmly than GA and MG in its binding pocket in kaouthiagin (Fig. **5A**). The docked conformation of GA (Fig. **5B**), MG (Fig. **5C**) was located at a similar position to that of the Ring C position of the docked PGG conformation (Fig. **5D**). The O10 atom of docked GA and MG conformations revealed an interaction with Zn^{2+} atom (1.99 Å and 2.67 Å, respectively) and H-bond interactions were also found with Gly117, His149, Glu150, His159, Leu174, Lys175, and Arg177. A hydrogen atom of a methyl group of the docked MG conformation formed an H-bond interaction with Val116. In the case of docked PGG conformation, the ring C revealed the same H-bond interaction as shown in the docked GA and MG conformations; i.e. Gly117, His149, Glu150, His159, Leu174, Lys175, and Arg177. The O28 atom of docked PGG conformation was found to interact with Zn^{2+} atom (2.67 Å). The other parts of the docked PGG conformations formed the H-bond interactions with Val116, Ile118, Ala119, Tyr120, Pro121, Ile157, His158, Asp160, Glu161, Ala162, and Lys176. Taken together, these phenolic compounds formed hydrogen bonds with the three active histidine residues in the conserved zinc-binding motif (HEbxHxbGbxHD) of the SVMPs from CR and NK venoms.

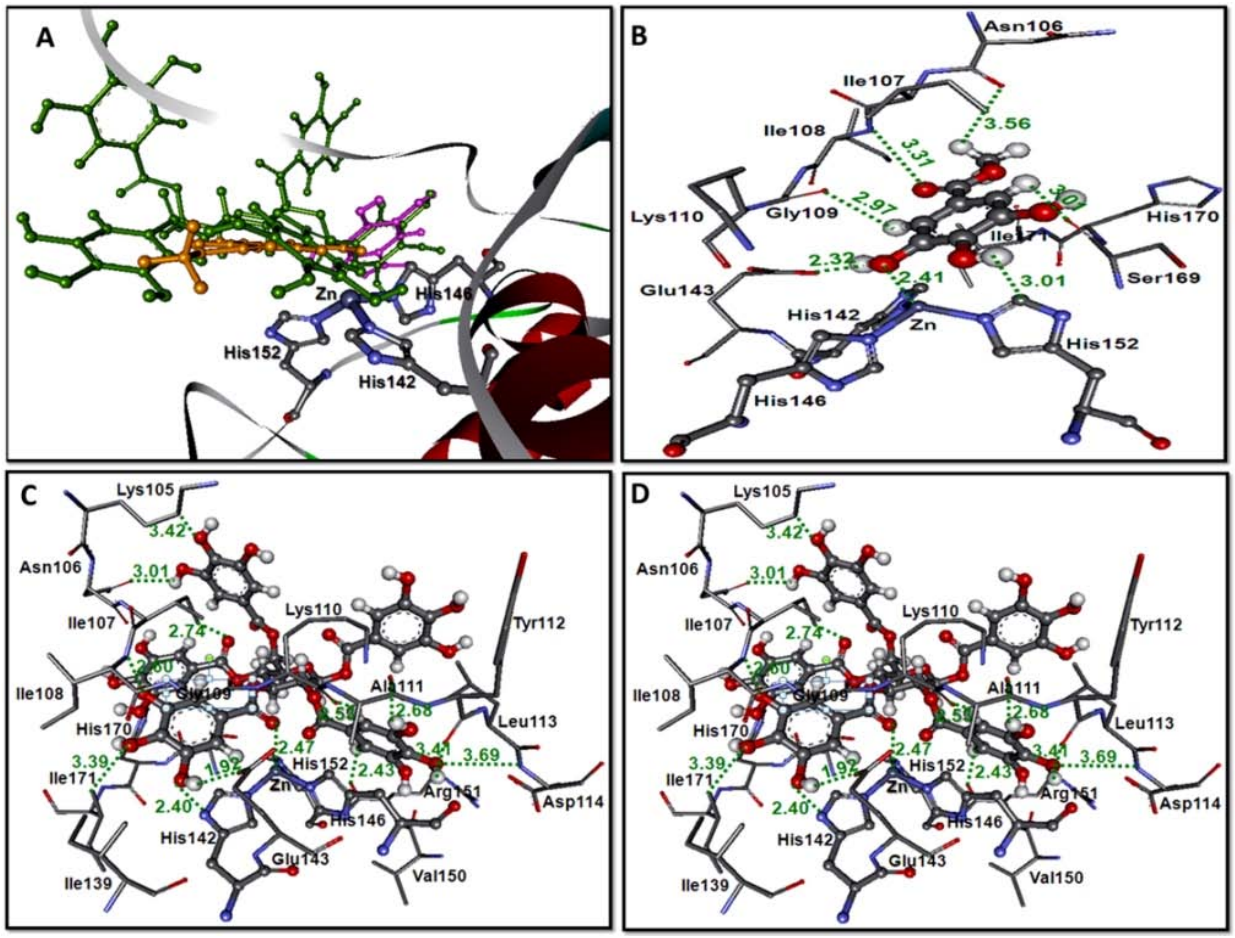


Fig. (4). (**A**) Docked conformation of ligand structures in the binding site of rhodostoxin (GA: Pink, MG: Orange and PGG: Green). (**B–D**) Distances (in Å) between residues in the rhodostoxin binding pocket and ligands: GA (**B**), MG (**C**) and PGG (**D**). (The figure has been adopted with permission from Molecules (MDPI), Ref; Pithayanukul *et al*., 2009).

These active histidine residues are also involved in the binding of the catalytically essential zinc ion, and by binding the inhibitors to these residues the catalytic efficiency of SVMPs can be changed [90].

Aung *et al.*, [91] studied the inhibitory potential of rosmarinic acid from *Argusia argentea* against the hemorrhage induced by *Trimeresurus flavoviridis* venom. RA significantly inhibited the hemorrhagic effect of crude venoms of *Trimeresurus flavoviridis*, *Crotalus atrox*, *Gloydius blomhoffii*, *Bitis arietans* as well as SVMPs, HT-b (*C. atrox*), bilitoxin 2 (*Agkistrodon bilineatus*), HF (*B. arietans*), and Ac1-proteinase (*Deinagkistrodon acutus*). The authors claim this as the first report of the anti-hemorrhagic property of RA, which greatly contributes to the anti-hemorrhagic efficiency of *A. argentea* against crude snake venoms and SVMPs. Chatterjee *et al.*, [92] isolated a small straight chain compound containing methyl and amide radicals (SNVNF) from the whole seed extract of *Strychnos nux vomica* (in low doses) and illustrated effective neutralization of *Daboia russelii* and *Naja kaouthia* venom induced lethality, hemorrhage, and defibrinogenating activities.

Sarkhel *et al.*, [93] isolated Di-iso-butyl phthalate from the root of *Emblica officinalis*, which have been reported to possess anti-snake venom property against viper & cobra venoms. The compound neutralized lethality, hemorrhage, coagulant activity, defibrinogenation, fibrinolysis, myotoxic activity and free radical generation induced by viper & cobra venoms. Further, the viper venom induced myotoxicity was antagonized by the compound as shown by the decreased levels of the myotoxicity marker enzymes CK & LDH.

Vale *et al.*, [94] isolated iso-quercitrin, myricetin-3-O-glucoside, catechin and gallocatechin from the aqueous extract of *Schizolobium parahyba* leaves and tested for hemorrhagic and fibrinogenolytic activities of *Bothrops* crude venoms and isolated SVMPs. The inhibitors neutralized the biological and enzymatic activities of *Bothrops* venoms and toxins isolated from *B. jararacussu* and *B. neuwiedi* venoms. Both gallocatechin and myricetin-3-O-glucoside are shown to be the potent inhibitors of hemorrhagic and fibrinogenolytic activities of SVMPs respectively. Gallocatechin also inhibited the myotoxic activity of both *B. alternatus* venom and BnSP-6 (Lys49 PLA_2 from *B. neuwiedi*). Recently Mahadeswaraswamy *et al.*, [95] demonstrated the

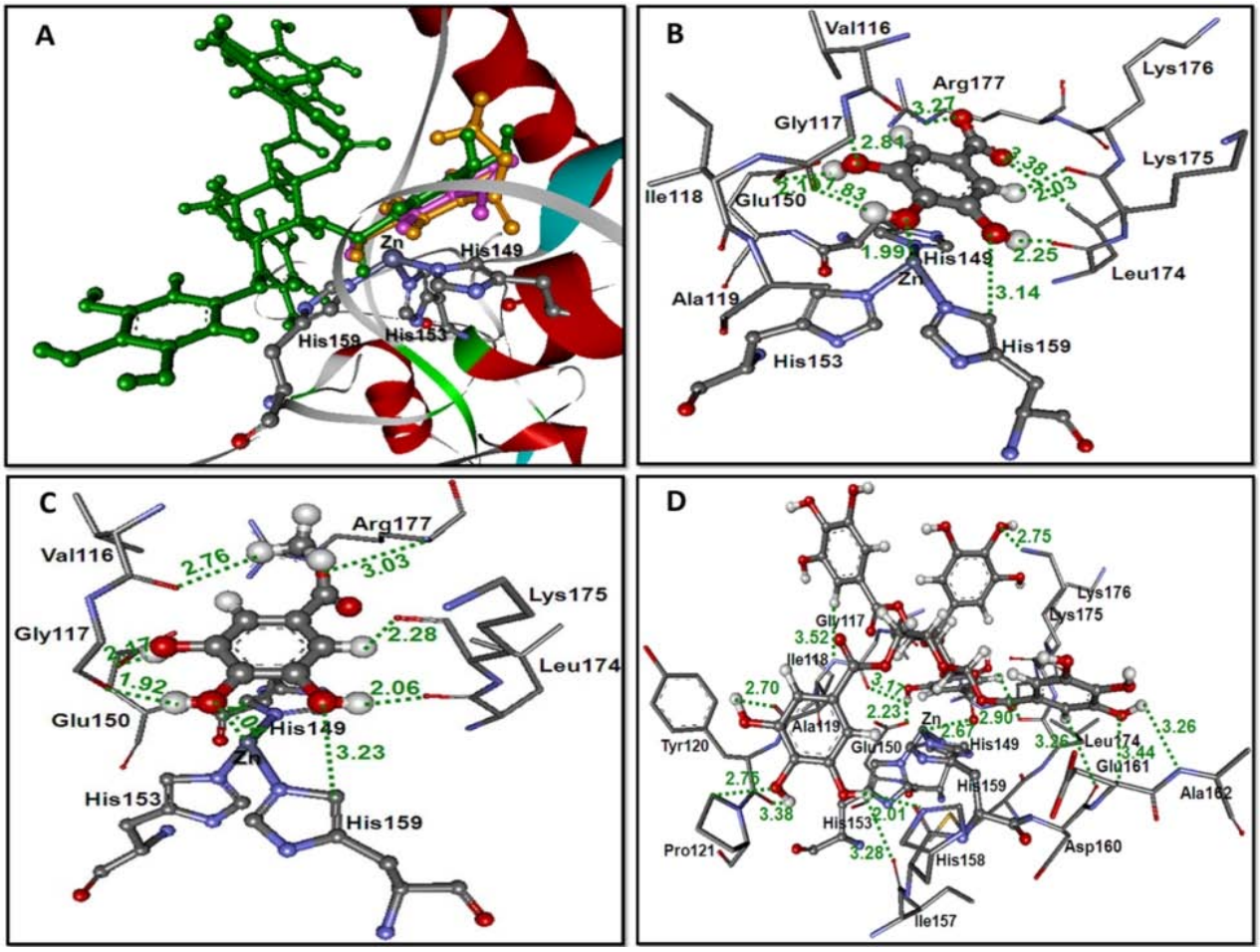


Fig. (5). (**A**) Docked conformation of ligand structures in the binding site of kaouthiagin (GA: Pink, MG: Orange and PGG: Green). (**B–D**) Distances (in Å) between residues in the kaouthiagin binding pocket and ligands: GA (**B**), MG (**C**) and PGG (**D**). (The figure has been adopted with permission from Molecules (MDPI), Ref; Pithayanukul *et al*., 2009).

anti-hemorrhagic property of the gallic acid (GA) against *Daboia russelli* (DR) venom and its purified hemorrhagic complex (HC). The hemorrhagic, edema forming, dermo and myonecrotic activities of both HC and DR venom are inhibited by GA. Further, GA also inhibited the degradation of ECM molecules such as collagen type IV, laminin and fibronectin by venom confirming the biochemical basis for GA, which inhibited local effects of HC and DR venom. Further Sunitha *et al*., [17] assessed the efficacy of N-acetyl cysteine (NAC) to inhibit gelatinase, hyaluronidase, hemorrhagic and defibrinogenating activities of *Vipera russelli* and *Echis carinatus* venoms. NAC inhibited these activities dose-dependently, but it did not inhibit the PLA_2, 5'nucleotidase, procoagulant and edema inducing activities of both the venoms. NAC inhibited the basement membrane degradation and accumulation of inflammatory leukocytes at the site of venom injection. The authors claims that observed inhibition of hemorrhage was likely due to zinc chelation as supported by spectral studies. Further, docking predictions suggested the role of -SH and -NH–CO-CH_3 groups of NAC in the inhibition of SVMPs and SVHYs.

Recently, Sunitha *et al*., [18] evaluated the inhibitory effect of 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-oxo-1,3-dihydroisobenzofuran-5-carbonitrile (DFD) on viper venom induced hemorrhagic activities. The synthetic drug effectively neutralized the hemorrhagic activity of the medically important viper venoms such as *Echis carinatus*, *E. ocelatus*, *E.c. sochureki*, *E. c. leakeyi* and *Crotalus atrox* in a dose dependent manner. In addition, DFD also inhibited the venom induced hemorrhage in independent injection experiment. The percentage inhibition of haemorrhage was found to decrease when the injection of DFD was delayed. The histological examinations revealed that the DFD effectively neutralize the basement membrane degradation and accumulation of inflammatory leukocytes at the site of *Echis carinatus* venom injection further confirms the inhibition of hemorrhagic activity. According to the docking studies, DFD binds to hydrophobic pocket of SVMP with the Ki of 19.26 x 10^{-9}(kcal/mol) without chelating Zn^{2+} in the active site (Fig. **6**). Taken together, the inhibition of hemorrhagic and myonecrotic activities of SVMPs by the different form of phytochemicals could be due to the chelation of divalent metal ions, which are essential for the catalytic activity. Phytochemicals may also interfere with the enzyme or enzyme-substrate complex formation by occupying critical binding sites either in enzyme or substrate.

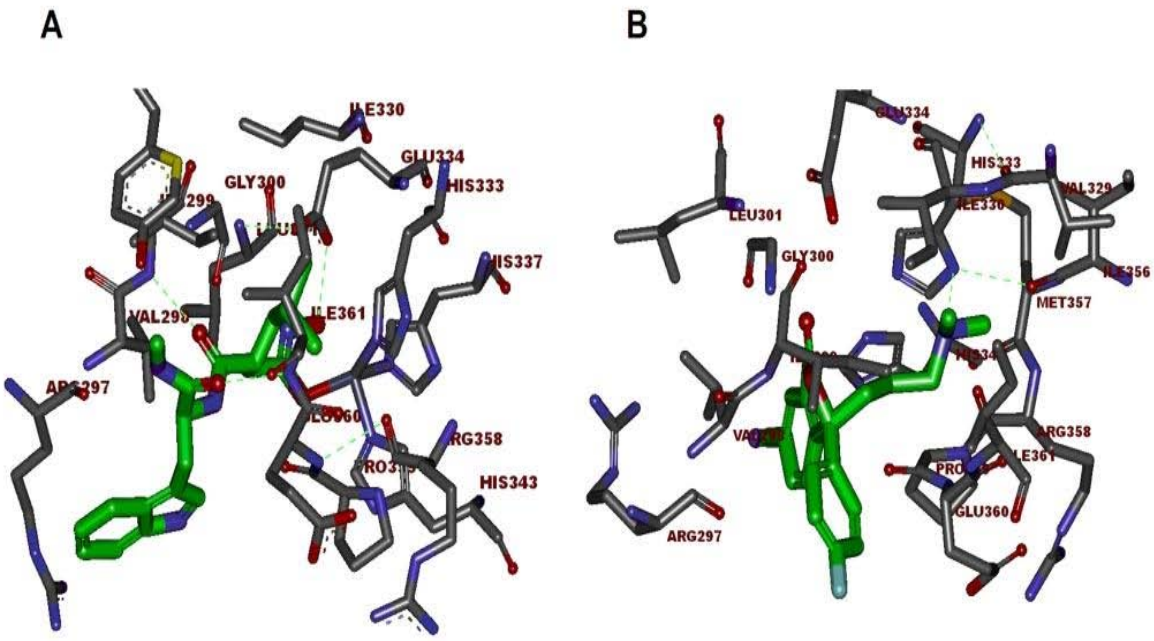


Fig. (6). Molecular docking structure of the hydrophobic binding pocket of bothropasin crystal structure (PDBID 2DW0). (**A**) Crystal structure of the hydrophobic binding pocket of bothropasin crystal structure showing molecular contacts with GM6001 (shown in green color). (**B**) Structural modeling of compound DFD (shown in green color) bound to the hydrophobic binding pocket of the bothropasin crystal structure. The DFD and GM6001 are shown as stick model. Hydrogen bonds are presented as dotted green lines. The grey, blue, and red color of the atoms refers to the carbon, nitrogen, oxygen, respectively. The data was rendered using discovery studio version 2.5. (The figure has been adopted with permission from journal, Ref; Sunitha *et al.*, 2011).

NEUTRALIZATION OF SVHYs

The biopolymer hyaluronic acid (HA) is a linear, megadalton, non-sulfated, glycosaminoglycan (GAG) present in ECM of soft connective tissues. Being a major component of ECM, HA interacts with the collagen fibers, protein filaments, growth factors, and hold water as well as metal ions [96]. It is composed of repeating disaccharide unit of D-glucuronic acid and N-acetyl-D-glucosamine linked through (β1-3 and 1-4) glycosidic linkages. The hyaluronidases are a class of endo-β-glycosidases distributed throughout the animal kingdom including various human organs and body fluids, external secretions of micro-organisms, leeches and in the venoms of snakes, scorpions, lizards, bees and other insects [40, 44]. The enzymatic degradation of HA is mediated through the co-ordinated activity of three different enzymes. The initial endoglycosidase activity on intact HA generates oligosaccharides of different chain length which become substrate for the two exoglycosidases namely the β-glucuronidase and β -N-acetyl hexosaminidase enzymes [97]. HAases and HA system is concerned with various biological functions ranging from embryogenesis to aging such as migration, adhesion, proliferation and differentiation of cells, immune surveillance, inflammation, wound healing, multi-drug resistance, angiogenesis, malignant transformation and water homeostasis and visco-elasticity of ECM [16, 40].

SVHYs are rightly referred to as a 'Spreading factor' as they facilitate easy diffusion of systemic toxins from the site of bite into general circulation. Rapid hydrolysis of mega structure HA into fragments of varied molecular size resulting in the decreased viscosity of the envenomed milieu aiding rapid diffusion of toxins into circulation, which would otherwise, diffused much slowly [8, 9, 44]. Further, the degraded end products of HA with high molecular mass are reported to be anti-angiogenic, anti-inflammatory and immunosuppressive, while low molecular mass fragments are pro-inflammatory, immune-stimulatory and angiogenic leading to complications [39]. In addition, during snakebite the bite site becomes the depot of venom, and rapid delivery of venom into the circulating blood is critical. The activity of SVHYs not only damages the tissue at the bite site, but also facilitates easy diffusion of systemic toxins into the circulation resulting in systemic toxicity. This limitation could be overcome through characterization of natural compound that can neutralize locally acting enzymes/toxins, thereby complementing the action of anti-venins. Thus inhibition of these spreading factors may result in slower delivery of target specific toxins into the circulation and hence would take longer time to attain the effective dose to dysfunction the target organs, as compared in absence of their inhibition [2, 16, 43, 44]. This would affect at least in two ways, first, it would widen the time gap between the bite and the anti-venom administration, secondly it would also reduce the anti-venom load to achieve effective neutralization and hence the side effects of the therapy. Several studies documented SVHYs inhibitors of different chemical forms such as proteins, and plant derived bioactive components including alkaloids, antioxidants, polyphenols, flavonoids, and terpenoids (Table **3**).

Machaiah *et al.*, [98] isolated a glycoprotein (WSG) SVHYs inhibitor (29 kDa) from *Withania somnifera* that could inhibit the SVHYs activity of Indian cobra (*N. naja*) and *Russel's viper* (*Daboia russelii*) venoms dose dependently. WSG inhibited the SVHYs and PLA_2 activities and prolonged the survival time of experimental animals injected with *N. naja* venom perhaps through preventing quick diffusion of the systemic toxins into the circulation.

Girish and Kemparaju, [42] showed the inhibitory action of plant alkaloids such as aristolochic acid, ajmaline and reserpine against the *N. naja* venom HAase, NNH1 activity. Partial inhibition was observed by ajmaline and reserpine while aristolochic acid completely inhibited the activity through non-competitive mechanism. The specto-fluorophotometer analysis confirmed the interaction of aristolochic acid with SVHYs in which the aristolochic acid quenched the pronounced fluorescence intensity of SVHYs in a dose dependent manner. It was clear from the studies that the aristolochic acid interacted with exposed tyrosine and tryptophan residues of the SVHYs rather than binding to the catalytic site. Aristolochic acid also inhibited SVHYs-potentiated toxicity of a myotoxic PLA_2 and a hemorrhagic complex of *D. russelli* venom apart from inhibition of the spreading property of the SVHYs. Furthermore, aristolochic acid also increased the survival time of experimental animals injected with *N. naja* venom [43].

The flavonoids have long been recognized and well-established anti-inflammatory, anti-oxidant, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral, and anti-carcinogenic properties [99, 100]. Extensive research studies have confirmed the potent inhibition of SVHYs by the structurally related flavonoids. Initially, Rodney and coworkers (1950) showed the inhibitory effect of a series of flavonoids on HAase and other related enzymes. Kuppusamy and Das, [101] analyzed the inhibitory efficacy of 12 flavone derivatives on the HAases of bee, scorpion, rattlesnakes and Malayan cobra venoms. Among the tested compounds, apigenin, kaempferol, luteolin, myricetin, phloretin (chalcone) and quercetin were found to inhibit partially the rattlesnakes and Malayan cobra venoms to the extent of 89% at 250μM concentration. The authors finally conclude that the flavones, flavonols and chalcones possess the general ability to inhibit SVHYs. The inhibition of *Crotalus adamenteus* venom HAase supported the above inhibition study further by apigenin, kaempferol, luteolin, quercetin and tannic acid [102]. In addition, Girish and Kemparaju, [42] reported the complete inhibition of *N. naja* venom HAases NNH1 enzyme by quercetin while, 89% and 94% inhibition respectively by flavone and tannic acid.

Dietary antioxidants, including polyphenols, are considered important because of their potential protective role in various pathological conditions. Antioxidants are reported to exhibit a wide spectrum of biological activities, including anti-carcinogenic, anti-inflammatory and anti-viral actions. In addition to enzymatic degradation of HA at the bitten site, non-enzymatic degradation by free radicals cannot be ignored. Therefore, anti-oxidants with HAase inhibition not only reduce the local tissue damage but also prevent oxidative stress induced cell death. Girish and Kemparaju, [42] screened inhibitory property of known antioxidants such as NDGA (nordihydroguaiaretic acid), curcumin, n-propyl gallate, BHT (butylated hydroxytoluene), chlorogenic acid, and Vit C against *N. naja* venom HAase. The maximum inhibition was observed with NDGA and curcumin while other compounds showed varied extent of inhibition. Recently, Mahadeswaraswamy *et al.*, [103] have demonstrated the dose dependent inhibition of SVHYs, (DRHyal-II) from *Daboia russelli* venom by mimosine, (β-3-(3-hydroxy-4-oxopyridyl) α-amino-propionic acid). The fluorescence emission and CD spectral studies confirmed the inhibition was due to the formation of DRHyal-II-mimosine complex that resulted in significant structural changes at secondary and tertiary levels. Some studies demonstrated electrostatic interaction between the functional groups of polysaccharides and COO^- groups of HA lead to the formation of inhibitor-HA complex that limits the substrate availability for the enzyme, resulting in HAase inhibition. Though the exact mechanism of action of SVHYs inhibition are not clear it can be concluded that these phytochemicals can either result in the formation of a complex or can bind to the active site or the sites other than active site thereby leading to the effective inhibition of the SVHYs.

Table 3. List of Plant Metabolites Effective against SVHYs

Chemical Form	Name of the Compounds	References
Protein	Glycoprotein (WSG)	[98]
Amino acid derivative	Mimosine, N-acetyl cysteine	[103, 17]
Alkaloids	Aristolochic acid, Ajmaline, Reserpine	[42]
Flavonoids/terpenoids	Flavone, Querticin, Myricetin, Apigenin, Kaempferol, Luteolin, Phloretin	[101]
Antioxidants/polyphenols	Catechin, Ascorbic acid, BHT, Nordihydroguaiaretic acid, Curcumin, N-propyl gallate, Chlorogenic acid, Tannic acid	[42]
Anti-inflammatory drugs	Dexamethasone, Indomethacin, Sodium cromoglycate, Salicylates, Sodium aurothiomalate	[16]

Table 4. List of Synthetic Compounds Effective against the Major Hydrolytic Enzymes of Snakevenom

Enzyme	Mane of the Compound	Snake genera
SVMPs inhibitors	1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-oxo-1,3-dihydroisobenzo-furan-5-carbonitrile	*Echis carinatus*; *E.c. sochureki*; *E.c. leakeyi*; *E. oceollatus*; *Crotalus atrox*
	Elaidoylamide	*Vipera ammodytes meridionalis*;
	d2-isoxazoline	*Vipera russelli*
	Indoles, Azetidinones, Piperazines, Isoxazolidines, Isoxazolines, Diazepinones, Acenaphthenes	*Viper venom*
	Clodronate; Doxycycline; Batimastat	*Bothrops asper*
	Marimastat, AG-3340, CGS-270 23A; Bay-12 9566; EDTA; TPEN; BAPTA; CaNa2EDTA; SCH 47890	*Echis ocellatus*; *Bothrops asper*; *Crotalus atrox*
	N-(2-furoyl)-(Z)-alpha,beta-didehydroleucyl-L-tryptophan 2	*Crotalus Adamanteus*
PLA_2 inhibitors	Benzoyl phenyl benzoate derivates	*Naja melanoleuca*; *Trimeresurus flavoviridis*
	1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-oxo-1,3-dihydroisobenzo-furan-5-carbonitrile	Crotalus atrox*;* *E.c. leakeyi*
	Trimethoxyphenyl isoxazolidine derivatives	*Vipera russelli*
	2-carbamoylmethyl-5-propyl-octahydro-indol-7-yl-acetic acid	*Daboia russelli pulchella*
	Prenylated and benzylated pterocarpans	*B. jararacussu*
	Suramin	Bothropstoxin-I (BthTX-I)
	Oxazolidinone Phospholipid Analogs	*Naja naja atra*; *Agkistrodon halys blomhoffii*
	Phospholipid analog with an oxazolidinone ring	*Naja naja atra*; *Agkistrodon halys blomhoffii*
	Thioether amide containing phospholipid analogs	*Naja naja naja*
	Alkylbenzoylacrylic acids	Viper venom
	1,2,3-triazoles	*Lachesis muta*
SVHYs inhibitors	Benzoyl phenyl benzoate derivates	*N. melanoleuca*; *Vipera russelii*; *Naja naja*

Apart from natural compounds, several synthetic drugs/ molecules have been screened against PLA2, SVMPs and SVHYs. Table **4** enlists the synthetic drugs effective against the enzymes associated with local manifestations.

FUTURE PERSPECTIVE

Although antivenins are life saviors, their limitations have roused a keen interest across the venom toxinologists and medical practitioners. The geographical and seasonal variability among the species and the deficient of competent production of anti venoms has rendered complications in snakebite treatment strategies. Since from last three decades, attempts have been made targeting pathological and therapeutic aspects of venom toxins and their specificity and mode of action. Understanding the specificity and variability, structure-function relativity of toxins and the associated antigenicity would significantly be useful in management of individual snakebites. In addition, toxin specific production of monoclonal antibodies would definitely contribute a remarkable revolution in the efficacy of anti-venom and make more effective than ever before. Unfortunately, the existing antivenin therapy is a failure in effective management of local effects at the bitten site as well as post-medicated complications usually develops after the hospital stay. Moreover, this has been legibly ignored by both researchers and medical practitioners. In addition, envenomation of target specific toxins may also induce oxidative stress to resident cells to secrete pro-inflammatory mediators leading to the oxidative burst and cell death. Administered antivenin can only neutralize circulatory target specific toxins but not the oxidative damage of resident cells as well as the altered inflammatory mediators that prevail even after antivenin administration. In cosequence, the victim may develop an altered blood pressure either due to oxidative stress or psychological status. In view of this, development of auxiliary

therapies towards the management of local effects at the bitten site and secondary complications are critically essential.

The medicinal plant products/isolated compounds and their generics are being considered to be the potential candidates as an auxiliary to antivenin therapy in particular dealing with the local toxicity. Recent studies have publicized the mechanistic action of certain phytochemicals, which either chelate metal ions in particular Zn^{2+} and Ca^{2+} of the individual toxin or block the receptors. The structure-computational study deduced that many phytochemicals could directly interact with active site amino acids of the toxin and neutralize its toxicity. The advantage of plant metabolites is that they are easily available, stable and could neutralize wide range of venom antigens, more importantly many of these compounds have the anti-venom potentiating actions that should be explored for the development of future therapeutics. Further, the combination of these two antidotes may find a suitable alternative for the ophidian accidents. Future studies targeting the three dimensional structure of individual enzymatic and non enzymatic toxins along with molecular modeling and molecular dynamics might offer a fresh path to design classic precise inhibitors. However, an approach with high degree of strategy has to be made to develop a single molecule which can be potent enough to inhibit ECM degrading enzymes thereby could stop local toxicity absolutely. This could also be exploited in the treatment of various multifactorial pathophysiological disorders like inflammation and cancer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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