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Antisnake Venoms and their Mechanisms of Action: A Review

I. Sani[1*], R.A. Umar[2], S.W. Hassan[2], U.Z. Faruq[3]
[1]Department of Biochemistry, Kebbi State University of Science and Technology, Aliero, Nigeria
[2]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Department of Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

Review Article

***Corresponding author**
I. Sani

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Abstract: Snakebite is a major socio-medical problem affecting many communities globally, especially African and Asian countries. It is still depending on the usage of antisera as the major source of treatment, which has its limitations. In many rural communities, health care facilities are inadequate and victims of snakebites mostly depend on traditional healers for herbal antidotes as an alternative treatment. This review has focused on the mechanisms of action of the animal derived anivenoms (antisera or antivenins) as well as those derived from plant resources (phyto-antivenoms). Antigen-antibody reaction is the basic mechanism of snake venom neutralization by the antivenins, but for the phyto-antivenoms, there are many hypotheses proposed on how the herbal compounds neutralize the toxic venom constituents. These include; protein precipitation, enzyme inactivation, chelation activity, adjuvant action, antioxidant activity, combination of these activities, etc. The review might be a stepping stone in establishing an effective and widely acceptable future therapy against snakebite treatment and management.
Keywords: Snake, Venom, Antivenin, Phyto-antivenom, Toxicity.

INTRODUCTION

Snakes

Snakes are elongated, legless, carnivorous reptiles of the suborder Serpentes that can be distinguished from legless lizards by their lack of eyelids and external ears. Like all squamates, snakes are ectothermic, amniote vertebrates covered in overlapping scales [1]. They are ubiquitous creatures that vary in their morphologic characteristics and potential for envenomation.

The non-venomous snakes belong to the family Colubridae (e.g. grass snake) while others known to cause severe envenomation belong to Elapidae (such as cobras, kraits, mambas and coral snakes), Viperidae (such as American rattlesnake, russels viper and aders) and Hydrophidae (such as sea snake) [2]. Three species; black-necked spitting cobra (*Naja nigricollis*), carpet viper (*Echis ocellatus*), and puff adder (*Bitis arietans*), belonging to the first two families, are the most important snakes associated with envenoming in Nigeria [3].

Snake Venoms

Snake venoms are highly modified saliva containing zootoxins used by snakes to immobilize and digest prey or to serve as a defense mechanism against a potential predator or other threats. The venom produce by the snakes' venom gland apparatus is delivered by an injection system of modified fangs that enable the venom to penetrate into the target [4].

Snake venom is composed of complex mixture of active substances, mainly peptides and proteins which interfere with the course of several biological processes in humans [5]. The components include among others; phospholipase A_2, acetylcholinesterases, L-amino acid oxidases, serine proteinases, metalloproteinases, hyaluronidases, phosphomono- and phosphodi-esterases, nucleotidases, myotoxins, haemorrhagins, coagulants, cardiotoxins, cytotoxins and neurotoxins [6]. Snake venom also contains inorganic cations such as sodium, potassium, calcium, magnesium and small amounts of zinc, nickel, cobalt, iron and manganese [7]. Zinc is necessary for anticholinesterase activity. Calcium is required for activation of enzyme like phospholipase [8].

Different toxins from snake venom exhibit different modes of action. Furthermore, venoms from different species vary greatly in their toxin composition [4]. Some distinct types of venom toxins that act differently include: *Proteolytic toxins* (found in cobras and mambas venom) distort the molecular structure of the affected body muscles causing necrosis and disability; *Cardiotoxic toxins* (found in the venom of *Naja* species) specifically affect the heart by impairing muscle contraction causing the heart to beat irregularly or completely stop beating; *Neurotoxic toxins* (found in

the venom of cobras, mambas, sea snakes, kraits and coral snakes) act on the nervous system and brain resulting in *tetany* where the nerve stays stimulated, causing incontrollable muscle contractions that can lead to death; *Dendrotoxic toxins* (found in cobras and mambas venom) inhibit neurotransmissions by blocking the exchange of positive and negative ions across the neuronal membrane leading to no nerve impulse, thereby paralyzing the victim; *Hemotoxic venoms* (found in the venom of Viperidae family members) cause hemolysis, or destruction of red blood cells (erythrocytes) and death. These differences in composition of venom depend upon the species, geographical location, habitat, climate, age etc. Hossain *et al*., and mode of action of venoms from different snakes make it very difficult to find single universal antivenin [9].

Snake venom is broadly classified as neurotoxic and haemotoxic. Cobras, Kraits and sea snakes have neurotoxic venoms. Vipers contain haemotoxic venom [7].

Proteins constitute 90-95% of venom's dry weight and they are responsible for almost all of its biological effects. Among hundreds, even thousands of proteins found in venom, there are toxins, neurotoxins in particular, as well as nontoxic proteins (which also have pharmacological properties), and many enzymes, especially hydrolytic ones [10]. Enzymes (molecular weight 13-150 KDa) make-up 80-90% of viperid and 25-70% of elapid venoms: digestive hydrolases, L-amino acid oxidase, phospholipases, thrombin-like pro-coagulant, and kallikrein-like serine proteases and metalloproteinases (hemorrhagins), which damage vascular endothelium [11]. Polypeptide toxins (molecular weight 5-10 KDa) include cytotoxins, cardiotoxins, and postsynaptic neurotoxins (such as α-bungarotoxin and α-Cobratoxin), which bind to acetylcholine receptors at neuromuscular junctions. Compounds with low molecular weight (up to 1.5 KDa) include metals, peptides, lipids, nucleosides, carbohydrates, amines, and oligopeptides, which inhibit angiotensin converting enzyme (ACE) and potentiate bradykinin (BPP). Phosphodiesterases interfere with the prey's cardiac system, mainly to lower the blood pressure [12].

Phospholipase A2 causes hemolysis by lysing the phospholipid cell membranes of red blood cells. Amino acid oxidases and proteases are used for digestion. Amino acid oxidase also triggers some other enzymes and is responsible for the yellow colour of the venom of some species. Hyaluronidase increases tissue permeability to accelerate absorption of other enzymes into tissues. Some snake venoms carry fasciculins, like the mambas (Dendroaspis), which inhibit cholinesterase to make the prey lose muscle control [13].

Thus, snake venoms typically contain neurotoxins, but they also often include enzymes that promote various hydrolysis reactions. The neurotoxins carry out the task of immobilizing the victim by interrupting the ability of the nerve cells to stimulate muscle movement. Hydrolysis helps make the tissues of the victim easier for the snake to digest if it is eaten. These hydrolytic enzymes may include molecules capable of breaking down collagen and phospholipids as well as other enzymes [14].

Mechanisms of Snake Venom Toxicity

Neurotoxic Venom

This venom causes death by paralyzing respiratory muscles and asphyxiation (Asphyxia: condition produced by occlusion of the airway e.g.. during strangling; pronounced stimulation of respiration with violent efforts; blood pressure and heart rate rise sharply, blood pH drops, nor-epinephrine secretion is greater than epinephrine and eventually respiratory efforts cease, blood pressure drops & heart rate slows. Death occurs due to cardiac arrest if artificial respiration is not started within 4-5 mins [15].

The action of cobrotoxin arises from its ability to bind strongly to the receptors in postsynaptic neurons. In order for one nerve cell to signal a neighbor, it releases neurotransmitters that diffuse across a small gap called the synapse. The nerve on the other side of the synapse (the postsynaptic neuron) has proteins that are specifically geared to sense the presence of these neurotransmitters. If these receptors are blocked, the signal is not passed on, and the nerve cell function is disrupted. Cobrotoxin is able to disrupt a specific type of receptor that is sensitive to acetylcholine. Once this polypeptide binds to the receptor, it is not released, so the nerve cell loses its ability to signal and the muscles it is supposed to trigger do not function. If the muscle that stops working is the diaphragm, the animal will not be able to breathe and it essentially suffocates [16].

Haemotoxic Venom

This venom causes death by causing tissue destruction and widespread haemorrhage (bleeding from some internal organ/part of the body associated with defective clotting mechanism in the body) [8].

Over the years, a number of toxins that affect blood circulation have been isolated and characterized from various snake venoms [17]. Some of them affect platelet aggregation, whereas others affect blood coagulation. Venom proteins affecting blood coagulation can functionally be classified as pro-coagulant or anticoagulant proteins on the basis of their ability to shorten or prolong the blood-clotting process [18]. Pro-coagulant proteins are either serine proteinases or metalloproteinases. They induce blood coagulation either by specifically activating zymogen, one of the blood coagulation factors, or by directly

converting soluble fibrinogen into an insoluble fibrin clot [18].

Snake venom metalloproteinases are endoproteolytic enzymes. Their catalytic activity is dependent on Zn^{2+} ions. On the basis of size and domain structure characteristics, they are classified into P-I, P-II, P-III and P-IV classes [19]. P-I proteinases contain only a metalloproteinase domain, P-II proteinases contain metalloproteinase and disintegrin domains, P-III proteinases contain metalloproteinase, disintegrin-like and cysteine-rich domains, and P-IV proteinases contain the P-III domain structure plus lectin-like domains connected by disulphide bonds. Most metalloproteinases are fibrinogenases and they release peptides from the C-terminal of fibrinogen. They are classified into α- and β-fibrinogenases on the basis of their specificity for the α or β chain of fibrinogen [20]. α-Fibrinogenases inhibit blood coagulation, because truncated fibrinogen does not form as strong a fibrin clot as the native fibrinogen. Thus the subtle physical destruction leads to the anticoagulant action of metalloproteinases.

Snake venom serine proteinases, in addition to their contribution to the digestion of prey, affect various physiological functions. They affect platelet aggregation, blood coagulation, fibrinolysis, the complement system, blood pressure and the nervous system [21]. Among the serine proteinases, only protein C activators exhibit direct anticoagulant effects. Physiologically, the zymogen of protein C circulating in the blood is activated by thrombin. This activated protein C degrades FV/FVa and FVIII/ FVIIIa, and releases a tissue-type plasminogen activator. It also stimulates fibrinolysis through its interaction with plasminogen activator inhibitor [22].

Snake venom toxins that prolong blood coagulation are proteins or glycoproteins with molecular masses ranging from 6kDa to 350kDa. These factors inhibit blood coagulation by different mechanisms. Some of these anticoagulant proteins exhibit enzymatic activities, such as PLA2 (phospholipase A2) and proteinase, whereas others do not exhibit any enzymatic activity (such as C-type lectin related proteins and three-finger toxins) [18].

SNAKEBITE ENVENOMATION

Snakebite 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 components [10]. The pathophysiology of snake envenomation includes both local and systemic effects. 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) [23]. 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 [24].

TREATMENT OF SNAKEBITE ENVENOMATION

Snakebite envenomation is frequently treated with parenteral administration of horse or sheep-derived antivenins aiming at neutralization of toxins [4]. However, antivenins have some disadvantages, thus limiting their efficient use. For example they can induce adverse reactions ranging from mild symptoms to serious anaphylaxis. Additionally, they do not neutralize the local tissue damage [25].

In many communities (especially rural), plant extracts have been used traditionally in the treatment of snakebite envenomation. Thus, vegetal extracts have been found to constitute an excellent alternative with a range of antisnake venom properties.

Serum-based Antivenin

Currently, serum-based antivenin is the only medically approved antidote against snakebite envenomation. It is usually pepsin refined F (ab) fragments of IgG purified from the serum of a horse or sheep that has been immunized with the venom of one or more species of snakes [4]. Antivenin neutralizes the venom of a particular species (monovalent or monospecific) or various different species (polyvalent or polyspecific). The antibodies against a particular species may also neutralize the venom of a closely related species (paraspecific activity) [24].

Monospecific antivenins are antivenoms that are intended for use in envenoming due to a single species of snake or a few closely related species whose venoms show clinically effective cross-neutralization [26]. It is practically possible only when there is a very high prevalence of a single species of snakes in the desired region, but most of the countries are inhabited by more than one medically relevant species of snakes, where use of polyspecific antivenins is highly recommended [27].

The polyspecific antivenins are produced by immunizing an animal with venoms of more than one species of snakes of high medical relevance to the concerned geographic area [28]. Other methods of production includes i) immunizing individual animals with venom of a single species and then mixing the

various hyper immune plasma for fractionation and ii) mixing appropriate quantities of relevant purified antivenins before formulation [29]. These polyspecific antivenins should be promoted whenever feasible technically, as they offer clinical advantages like better usefulness than monospecific antivenins. Their use reduces the need for identification of snakes prior to initiation of antivenom therapy and simplicity in logistics provides great advantages [26].

Mechanism of Action of Antivenin

The ability of antigens to induce an immune response is called immunogenicity. Thus, snake venoms have two types of components: (a) Immunogenic components, which are proteins that constitute more than 98% of the venom dry weight and include toxins able to induce relevant toxic effects as well as non-toxic proteins, and (b) non-immunogenic components, such as amino acids, nucleotides, carbohydrates, lipids and biogenic amines, which constitute less than 2% of the venom dry weight and, in general terms, are devoid of toxicity [30].

Depending on how the snake venom toxins are presented to the immune system, different antibody responses can be obtained. The generation of an antibody response effective to recognize and neutralize toxic proteins of venoms is the key element in the production of effective antivenins in animals [31].

There are several types of antibodies and antigens, and each antibody is capable of binding to only one specific antigen. The specificity of the binding is due to specific chemical constitution of each antibody. The antigenic determinant or epitope is recognized by the paratope of the antibody situated at the variable region of the polypeptide chain. Antigens are bound to antibodies through week and noncovalent interaction such as electrostatic interaction, hydrogen bond, Van der Waals forces and hydrophobic interaction [32].

Snake venoms are composed by proteins whose antigenicity and abundance changes from one species to another. However, some antigenic similarities are conserved among phylogenetically related species. Therefore, antibodies rose by immunization with particular snake venom, in addition to reacting with its homologous toxins, can cross-react with some toxins present in heterologous venoms and may neutralize their toxic effects [33]. The specificity of antivenin antibodies towards a restricted group of venoms is a limitation of the immunotherapy of snakebite envenomation [30].

Venom-antivenin interaction is an antigen-antibody reaction, and this reaction is the fundamental reaction for neutralizing toxins present in snake venom. In the blood, venom toxins are specifically with high affinity bound by antibodies (antivenin) to form antigen-antibody complex. These complexes are then transported to cellular system where they can be destroyed or deactivated [34].

Antibodies possess at least two antigen-binding sites and most of the venom antigens have at least two epitopes (antigenic determinants). The antibodies cross-link antigens forming large aggregates of antibody and antigen referred to as immune complexes which are more readily phagocytized than are free antigens. However, snake venom reacts with surface-bound antigens of the cells and forms the immune complex. This process of agglutination of venom on the receptor applies to most snake venom toxins [35].

Once neutralized, the toxin-antibody complex is either unable to attach to receptor sites on host target cells, unable to enter the cell, or it is ingested by phagocytes. Elimination process of venom complexes in the presence of antivenin is much slower than that of free venom in the absence of antivenin, that is to say, most of these complexes are eliminated by phagocytosis than the renal route [35].

Herbal Antivenom

Due to inadequate health care facilities especially in rural areas, people largely depend on alternative treatment by traditional healers who have knowledge based on ancient culture, ethnic practices and herbal antidotes. The plant kingdom provides an inexhaustible source of various herbal compounds with pharmacological potential [36]. A plethora of medicinal plants, available locally are used widely by traditional healers in the form of plant sap, pastes, decoctions, powders and pills in the treatment of snakebite envenomation [37].

Mechanisms of Action of Herbal Antivenom

It was reported that herbal antivenoms neutralize the toxic venom constituents through either of the following: protein precipitation/inactivation [38], enzyme inactivation/inhibition [39], chelation activity [40], adjuvant action [41], antioxidant activity [42] and combination of these activities [41]. Among these, protein precipitation and enzyme inhibition are more acceptable [43].

Protein Precipitation

Various phytochemicals with protein binding properties, active against snake envenomations include flavonoids, polyphenols, saponins, tannins, terpenoids, xanthenes, quinonoids, steroids and alkaloids. These bind to toxic venom proteins there by inactivating them. They could also competitively block the target receptors [44]. Flavonoids like myricetin, quercetin and amenthoflavone isolated from *Davilla elliptica* and *Byrsonima crassa* leaves have antihemorrhagic activity against venom of *Bothrops jararaca* [45].

Sunitha *et al*., [13] investigated the molecular mechanism by which polyphenols from plant extracts interact with α-cobratoxin, a neurotoxin from the venom of certain *Naja* snakes, which causes paralysis by preventing the binding of acetylcholine to the muscle-type nicotinic acetylcholine receptor (nAChR). They evaluated the interaction between this toxin and some phenolic compounds, such as tannic acid, digallic acid, procyanidin dimer and procyanidin trimer, present in the extracts of some selected plants. Molecular modeling revealed the formation of hydrogen bonding between hydroxyl groups of tannins and amino acid residues surrounding their binding site to α-cobratoxin.

Enzyme Inactivation/Inhibition

Snake venom phospholipase A2 (PLA2), metalloproteases and hyaluronidases are the key enzymes involved in snake venom toxicity [12]. Thus, inactivation of these enzymes is generally considered to be the fundamental step in the management of snakebite.

Many plants with snake venom enzymes inhibitory effect, such as *Curcuma longa* (Zingiberaceae), *C. verbenacea* (Boraginaceae), *Hemidesmus indica* (Asclepiadaceae), *Areca cathechu*, *Crinum jagus*, *Cordia verbenacea*, *Quercus infectoria*, etc. were believed to contain high level of polyphenols [46]. Polyphenols such as tannins are specialized metabolites found in many plants species that have been shown to interact with enzymes from snake venom by non-specific binding to the proteins [47]. In relation to the mode of action, several studies have concluded that the inhibition of polyphenolic compounds on snake venom enzymes is due to the interactions between the enzyme and the hydroxyl groups present in this type of metabolites, through hydrogen bonds results in the formation of a stable complex [48]. However, the activity of polyphenol compounds may involve varying degrees of interactions such as hydrophobic connections mediated by aromatic rings.

It was reported that, high-resolution crystal structures of two complexes formed between a PLA2 isolated from the venom of *D. russelli pulchella* with two plant compounds; anisic acid [49] and atropine [50] indicated that networks of hydrogen bonds and hydrophobic interactions stabilized the complexes formed. Interactions with His48 and Asp49 were observed in both complexes.

Chelation Activity

Soares *et al*., [51] suggested that plant extracts have compounds that bind to divalent metal ions, which are required for some enzymatic activities. As the presence of proper metal ion coordination is a pre-requisite for the hydrolytic activities of PLA2 and metalloproteases, any metabolite that can weaken the enzyme-metal ion interaction will result in the inactivation of the hydrolytic activity.

Snake venom proteases majorly affect haemostasis and cause systemic hemorrhage. The mechanism involved in the inactivation of these proteases by plant extracts could be due to the chelating property of phenolic components present in plant extracts. It is reported that, phenolic compounds form hydrogen bonds and strongly bind to the histidine residues present in Zn^{2+} binding motifs of metalloproteases, resulting decrease in the hydrolytic activity of the enzyme [52].

PLA2 causes cardiotoxicity, myotoxicity, pre or postsynaptic neurotoxicity, edema, hemolysis, hypotension, convulsion, inhibition of platelet aggregation and anticoagulation [53]. Snake venoms are especially rich in group I (Elapidae) and II (Viperidae) PLA2s. These enzymes are Ca^{2+} dependent and hydrolyze the 2-acyl ester bonds of membrane glycerophospholipids producing free fatty acids and lysophospholipids which are key components of inflammatory process [53]. The mechanisms by which plants can act against the snake venom PLA2 have been extensively studied. Among them, the phytochemicals as catequines, flavones, anthocyanines and condensed tannins were related in their abilities in the chelation of the Ca^{2+} required for the catalytic activity of snake venom PLA2 [12].

It is known from literature data that phenolic compounds can complex metal ions only when they have suitably oriented functional groups in their structure [54]. The presence of 3'-4' and/or 7-8 *o*-dihydroxyphenyl (catechol) groups or 5-OH and/or 3-OH in conjugation with a C4 keto group in phenolic compounds structure is important for metal ions binding [55]. The chelating activity increases when galloyl moiety (3',4',5'-OH trihydroxybenzene) is present in phenolic compound molecule. When chemical structure of tannins is considered, it could be presumed that condensed tannins, which are catechin polymers, bind metal ions mainly to catechol groups, whereas hydrolysable tannins (derivatives of gallic acid) to galloyl groups [56].

Adjuvant Action

Alam and Gomes [41] reported that a compound 2-hydroxy-4-methoxy benzoic acid (2-OH-4-MeO benzoic acid) isolated from the root extract of an Indian medicinal plant; Sarsaparilla (*Hemidesmus indicus* R. Br.) neutralized the snake venom-induced pathophysiological changes through adjuvant effects and antiserum potentiation. The compound increased the antibody production in hyperimmunized rabbits as evident by the increased fold of venom neutralization (both lethal and hemorrhagic activity). It was found, however, that the compound, when injected into male albino mice, showed the activation of macrophages and lymphocytes [57]. The venom antigen, when injected along with the compound, increases lymphocyte response for increased synthesis of antibodies. The

other action of the compound is that it acts as an adjuvant triggering the retention of small venom antigen particles and help in the formation of antibodies. Antiserum raised with the compound along with viper venom, effectively neutralized the lethal activity induced by the venom of *Vipera russellii*, *Echis carinatus*, *Naja kaouthia* and *Ophiophagus hannah*. But the viper venom (alone) induced rabbit antiserum was found to protect the venom lethal effect of viper venom only and not that of Echis, Naja and Ophiophagus venoms. The compound along with viper-venom induced rabbit antiserum, neutralized the viper and Echis venom-induced hemorrhagic action, but when only viper venom-induced rabbit antiserum was used, it only neutralized the viper venom induced hemorrhagic action. These observations indicate that the compound acts as adjuvant thus triggering the high titre antibody which effectively neutralized both the viperid and elapid venoms.

The compound potentiated the venom neutralizing action of commercial snake venom antiserum. Higher degree of neutralizing of both Cobra and viper venom was achieved by the compound. It was suggested that the compound executed this effect through increased antiserum retention and venom antigen presentation for better neutralization. Thus the compound has a dual action in this phenomena, (a) showing its adjuvant effect and (b) increasing antiserum efficacy [41].

Antioxidant Activity

The lethality of snake venom is mainly attributed to its highly active enzymatic component, phospholipase A2 (PLA2) that hydrolyzes cellular phospholipids, resulting in the release of arachidonic acid [14]. Oxidative metabolism of arachidonic acid generates potentially toxic reactive oxygen species (ROS) including superoxide and hydroxyl free radicals [58. An imbalance between the excessive generation and poor removal of ROS causes lipid peroxidation leading to cellular damage [59]. PLA2 from snake venom has been implicated in multiple pathologies including hepatotoxicity and nephrotoxicity [60]. Snake envenomation is also accompanied by signs of inflammation and local tissue damage. Neutrophils and macrophages are induced to produce superoxide radical anion (O_2^-) which belongs to the group of reactive oxygen species and this reacts with cellular lipids leading to the formation of lipid peroxides and the observed necrosis [11].

Plants secondary metabolites such as vitamins (A, C and E), flavonoids, terpenoids, tannins, other polyphenols and some minerals (selenium) have the capability in neutralizing free radicals; hence they are valuable natural antioxidants that scavenge and remove oxygen free radicals, stabilize cell membranes [61] and act as immunomodulators [62]. These classes of compounds are known to be powerful antioxidants both in hydrophilic and lypophilic environments. They can prevent, stop or reduce oxidative damage as a result of PLA2 activity by selectively binding to the active sites or modify conserved residues that are critical for the catalysis of the PLA2 [47]. On the other side, vitamin E (α-tocopherol, an antioxidant molecule) decreased both enzymatic and inflammatory activities of an isolated PLA2. In this direction, it was also suggested that vitamin E has the ability to bind to the hydrophobic pocket of PLA2, inhibiting free access of substrate to the catalytic site [63].

Presence of inorganic cations in snake venom, such as, iron and zinc can generate highly reactive $OH^{\bullet}$ radicals by Fenton reactions and superoxide ($O_2^{\bullet-}$) by Haber-Weiss reaction [16] after snakebite envenomation. Chelating agents, which stabilize prooxidative transition metal ions by complexing them, are regarded as secondary antioxidants. Until now studies were conducted on the metal ions chelating activity of pure tannins (commercial products) of known structure. Lopes *et al.*, [64] reported antioxidant properties of tannic acid to result from forming stable complexes with Fe (II). Mila *et al.*, [65] noted that polyphenols (tannins) remove Fe (III) from other iron/ligand complexes.

CONCLUSION

Natural products have been recognized as the most significant source of new leads for pharmaceutical development. Most of the world's population use plants for medication and for the relief of signs and symptoms of snake envenomation. Plant extracts and isolated compounds have shown inhibitory activity against snake venoms and their purified toxins. Furthermore, these inhibitors not only reduce local tissue damage but also retard the easy diffusion of systemic toxins and hence increase survival time. The continuity of studies on the mechanism of action, as well as the safety and toxicity of these molecules will reveal their potential use in the development of new snakebite therapies.

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