Review Article

Snake Venom PLA₂, a Promising Target for Broad-Spectrum Antivenom Drug Development

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Snakebite envenomation is a neglected global health problem, causing substantial mortality, disability, and psychological morbidity, especially in rural tropical and subtropical zones. Antivenin is currently the only specific medicine for envenomation. However, it is restricted by cold storage, snakebite diagnosis, and high price. Snake venom phospholipase A₂s (svPLA₂s) are found in all kinds of venomous snake families (e.g., Viperidae, Elapidae, and Colubridae). Along with their catalytic activity, svPLA₂s elicit a wide variety of pharmacological effects that play a pivotal role in envenomation damage. Hence, neutralization of the svPLA₂s could weaken or inhibit toxic damage. Here we overviewed the latest knowledge on the distribution, pathophysiological effects, and inhibitors of svPLA₂s to elucidate the potential for a novel, wide spectrum antivenom drug targeting svPLA₂s.

1. Introduction

Snakebite envenomation is a critical public health problem and fieldwork hazard, causing high mortality and morbidity, particularly in tropical and subtropical regions. As most ophidian incidents occur in rural areas of developing countries, accurate statistical data concerning the number of victims is difficult to obtain [1]. As extrapolated by Chippaux, worldwide 5,400,000 people are bitten by snakes, 2,500,000 are envenomed, 125,000 die, and more than 100,000 individuals suffer from severe sequelae each year [2]. Unfortunately, snakebite was neglected by governments and international health agencies for a long time, even though the snake bite mortality rate is equivalent to one-fifth of the deaths from malaria worldwide and half of the deaths from HIV/AIDS in India [3]. In 2009 the World Health Organization (WHO) recognized snake bite as a neglected tropical disease [1]. Currently, antivenin is the only specific treatment towards envenomation. Although the immunized animal sera (mainly horse or sheep) presently used are highly effective, they are limited by a few drawbacks [4]. First, local tissue damage resulting from snake venom exposure, often leading to amputation, cannot be reversed by antivenin [4]. Furthermore, early and late adverse reactions to antivenin (e.g., anaphylaxis, pyrogenic reactions, and serum sickness) occur in some cases [5]. Additionally, access to antivenins is often limited. Some remote, rural communities where antivenoms are most needed cannot get adequate supplies, due to the lack of cold chain storage and other complex political reasons. Finally, most antivenoms are too expensive for the patient’s family in low-income countries [6].

Recently, the nonprofit French drug firm Sanofi Pasteur had ceased the production of Fav-Afrique, the most effective antivenin against Africa’s vipers, mambas, and cobras. This has resulted in a large-scale snakebite crisis in rural Africa [7]. This alarming situation demonstrates the need for antivenin replacements and new antivenom drug candidates. This review article focuses on snake venom phospholipase A₂s (svPLA₂s), a chemical family that is widely distributed in venomous snake species. Here we describe svPLA₂s, the antivenomination effects of their inhibitors, and the potential of being a common target for broad-spectrum antivenom drugs.

2. Characteristics of svPLA₂

Snake venoms are complicated mixtures, consisting of phospholipase A₂s, metalloproteases, C-lectins, serine proteases,
L-amino acid oxidases, disintegrins, and a few other compounds [1]. Most svPLA$_2$s hydrolyze glycerophospholipids at the sn-2 position of the glycerol backbone, freeing lysophospholipids, and fatty acids. svPLA$_2$s share 44–99% amino acid identity in their primarily structure, which results to high similarity in their tertiary structure [8]. Based on their size, location, function, substrate specificity, and calcium requirement, PLA$_2$s are classified into six families. svPLA$_2$ belongs to the secretory PLA$_2$(sPLA$_2$) family (groups IA, IIA, and IIB) [9–11]. Cobras and kraits, rattlesnakes, and Gaboon vipers have svPLA$_2$s in groups IA, IIA, and IIB, respectively [8]. There are also group IB enzymes which are mainly found in mammalian pancreas that have been reported in some snake venoms, such as Oxyuranus scutellatus [12], Pseudonaja textilis [13], and Micrurus frontalis frontalis [14]. These compounds are conserved in structure and have similar molecular masses (~10–20 kDa), 5–7 disulfide bonds, and analogous three-dimensional structures [15]. In Group I there are approximately 115–120 residues, 7 disulfide bonds (the unique disulfide linking residues II and 77), and G IIA has a characteristic surface loop between residues 63 to 67 called elapidic loop [11]. While G IB has a five amino acids residues (residues 62–67) extension termed pancreatic loop, some G IB snake venom PLA$_2$ even has an eight-residue propeptide segment in their mature state [13, 16]. In contrast, Group II has a C-terminal extension, the unique disulfide linking residues 50 and 137. GIIA have a 7-residue C-terminal extension and seven conserved disulfide bonds, while in Group IIB, the C-terminal extension is 6 residues, and only six disulfides remained in which a universally conserved 61–95 disulfide is lacking [11]. Furthermore, a new subgroup (Lys$_4$s PLA$_2$ homologues) can be created through mutation. Replacement of the 49th residue (asparagine) with lysine results in an inactive or weakly toxic PLA$_2$. This lysine residue can also interact with other amino acids in the “calcium-binding loop” resulting in the loss of calcium-dependent catalytic activity [17, 18]. Most svPLA$_2$s exist as monomers, but some exist in complexes, which mainly exhibit presynaptic neurotoxicity through combination of isoenzymes or other proteins [19].

3. PLA$_2$s Are Extensively Distributed in Snake Venom

Mackessy [20] analyzed crude venom from the main clades of venomous snakes via SDS-PAGE and found that svPLA$_2$s existed in almost every family (Figure 1). The highest amounts were found in Elapidae, Viperidae, and Hydrophiidae. The lowest were found in Colubridae (which is usually nonvenomous). Through the application of transcriptomics and proteomics, we gained a better understanding of venom composition and the pharmacological properties of the venom components [21]. Betzel et al. found that PLA$_2$s made up 32–59.8% in Viperidae snake venom [22]. However, Bungarus fasciatus venom was found to consist of up to 71% of PLA$_2$s [23]. Moreover, Gutiérrez and Lomonte found that the most lethal fractions in Micrurus fulvius (family Elapidae) were two PLA$_2$ molecules which represented 33.4% of the whole venom [24]. To date, more than 464 unique svPLA$_2$s have been recorded in UniProtKB database. What has been presented above indicates that PLA$_2$s are abundant and fatal toxins in most snake venoms.

4. svPLA$_2$s Have a Wide Spectrum of Pharmacological Effects

Despite producing lysophospholipids and fatty acid proinflammatory mediators, svPLA$_2$s also present a wide spectrum of pharmacological effects in victims, (i.e., neurotoxicity, myotoxicity, anticoagulant effects, cytotoxicity, cardiotoxicity, and edema, Table 1). The diverse toxic effects are tightly related to the multiple functional sites on the surface of svPLA$_2$s and their different binding receptors [25].

4.1. svPLA$_2$ Neurotoxicity. Neurotoxic svPLA$_2$s can block neuromuscular transmission in vertebrate skeletal muscles causing acute neuromuscular weakness and paralysis resulting in respiratory depression and death [55]. Neurotoxic svPLA$_2$s are mainly found in the Elapidae (kraits, elapids, and coral snakes) and Viperidae (vipers and rattlesnakes). Their toxicity varies greatly among species, ranging from 1μg/kg (Textilotoxin) to 380 μg/kg (HDP-2 from Viper nikolskii) [53]. Previous studies indicate that there is no correlation between toxicity and PLA$_2$ hydrolysis activity. svPLA$_2$ neurotoxicity affects presynaptic nerve terminals, so these compounds are commonly referred as presynaptic neurotoxins or β-neurotoxins (β-ntxs) [54]. β-ntxs are monomers or noncovalent complexes containing 2–5 subunits with at least one PLA$_2$ subunit. To our knowledge, all β-ntxs hydrolyze phospholipids, especially anionic lipids (e.g., phosphatidylserine, phosphatidic acid, and phosphorylated phosphatidylinositol) which are abundant in the cytosolic leaflets of organelles and the plasma membrane of eukaryotic cells [55]. svPLA$_2$s also bind to special tissue sites to achieve their neurotoxicity effects. The mechanism of svPLA$_2$ neurotoxicity is still under investigation.

4.2. svPLA$_2$ Myotoxicity. svPLA$_2$s can induce acute necrosis of skeletal muscle (myonecrosis) [56]. In the envenomation, this myonecrosis can potentially lead to permanent tissue loss or amputation [57]. svPLA$_2$ myotoxins are mainly found in venom from Elapidae, including sea snakes and Viperidae [58]. Depending on the venom, these svPLA$_2$s can elicit local or systemic myotoxicity. Local myotoxicity is mainly elicited by viperid venom. This damage is limited to the region where the toxin is injected and is often coupled with hemorrhaging, blistering, and edema [57, 59]. Systemic myotoxicity is elicited by elapid venom (i.e., some sea snake, terrestrial elapids). This causes muscle damage and a distinct increase of creatine kinase (CK) activity in plasma and is associated with renal failure and myoglobinuria [58]. Along with sharing a highly conserved structure, svPLA$_2$ myotoxins are tightly associated with neurotoxins. Both achieve a similar cellular lesion through membrane perturbation, cytotoxic Ca$^{2+}$ homeostasis imbalance, and cell degeneration [60]. Furthermore, some neurotoxic svPLA$_2$s (e.g., notexin and crotoxin) cause acute skeletal muscle necrosis, adding to systemic toxic effects (i.e., rhabdomyolysis) [60].
Residue 49 in myotoxic svPLA₂ is usually associated with PLA₂ enzymatic activity. Asp⁴⁹-PLA₂ are generally strongly catalytic whereas Lys⁴⁹ homologues are either not catalytic or weakly catalytic. There are also other amino acid substitutions, such as Ser⁴⁹, Arg⁴⁹, Asn⁴⁹, or Gln⁴⁹ [56]. The lysophospholipids released from phospholipid that hydrolyzed by Asp⁴⁹ PLA₂ usually cause skeletal muscle necrosis via direct disruption of membrane stabilization and/or indirect biophysical alteration of membrane [61]. The Lys⁴⁹ PLA₂ myotoxins are devoid of catalytic activity, existing

**Figure I:** SDS-PAGE profile of major venom components in the main clades of venomous snakes (adapted from [20]). (a) Families: Elapidae, subfamilies Elapinae, Laticaudinae, Hydrophiinae, and Colubrinae. (b) Family: Viperidae, and subfamilies: Crotalinae (C) and Viperinae (V). Ovals enclose some bands that are typical of protein families, based on published mass. (?) indicates hypothetical protein family or activity.
### Table 1: Features, toxicities, binding receptors, and enzymatic activity of snake venom PLA\(_2\)s.

| Name            | Snake species            | Structural features subtype\(^a\)                       | Toxicities                                      | Lethality in mouse (µg/kg)\(^b\) | Binding proteins in tissue\(^c\) | PLA\(_2\) activity (µmol/min/mg toxin)\(^d\) | Reference |
|-----------------|--------------------------|----------------------------------------------------------|-------------------------------------------------|----------------------------------|----------------------------------|---------------------------------------------|-----------|
| Neurotoxin      |                          |                                                          |                                                 |                                  |                                  |                                             |           |
| Crotoxin        | Crotalus durissus terrificus | Heterodimeric; A: IIa-sPLA\(_2\)-like B: IIa-sPLA\(_2\) | Neurotoxicity; myotoxicity; cardiotoxicity      | 60–240 (i.v.)                     | Crocalbin; CaM                   | 85                                          | [26]      |
| M\(_2\)PLA\(_2\)-I | Micrurus spixii          | Monomeric; IA-PLA\(_2\)                                 | Neurotoxicity; myotoxicity; antiplasmolidal activity; edema | n.d.                             | nAdR                             | Yes                                         | [27]      |
| Taipoxin        | Oxyuranus scutellatus    | Trimeric; α: IA, toxic; β: IA-sPLA\(_2\)-like; γ: IB-sPLA\(_2\); glycosylated | Presynaptic neurotoxicity; cytotoxicity          | 2 (i.v.)                          | M-sPLA\(_2\); R; NP; TCBP-49     | 0.4                                         | [28–30]  |
| Textilotoxin    | Pseudonaja textilis      | Pentameric; A, B and C are IA- sPLA\(_2\); D\(_2\), identical to Slinked IB-sPLA\(_2\); glycosylated | Presynaptic neurotoxicity                       | 1 (i.v.)                          | M-sPLA\(_2\)-R; CaM; PDI; FXα; 14-3-3 proteins | v.d. K\(^+\) channel | 3.2       | [13, 28, 31] |
| Ammodytoxin     | Viperidae                | Monomeric; IA-sPLA\(_2\)                                 | Presynaptic neurotoxicity                       | 21 (i.v.)                         | M-sPLA\(_2\)-R; CaM; PDI; FXα; 14-3-3 proteins | v.d. K\(^+\) channel | 280       | [32–35]      |
| β-Bungarotoxin  | Bungarus multicinctus    | Dimeric; A: IA-sPLA\(_2\); S-S linked to subunit B: BPTI-like | Presynaptic neurotoxicity                       | 19–130 (i.p.)                     | M-sPLA\(_2\)-R; CaM; PDI; FXα; 14-3-3 proteins | v.d. K\(^+\) channel | 61        | [36, 37]     |
| Notexin         | Notesthes scutatus       | Monomeric; IA-sPLA\(_2\) (Asp49)                         | Myotoxicity; presynaptic neurotoxicity; nephrotoxicity | 17 (i.v.)                         | n.d.                             | 1390                                        | [38, 39]  |
| Myotoxin        |                          |                                                          |                                                 |                                  |                                  |                                             |           |
| Myotoxin III    | Bothrops asper           | Dimeric; IA-sPLA\(_2\) (Asp49)                          | Myotoxicity; anticoagulant; edema               | 470 (i.v.)                        | n.d.                             | 750                                          | [40]      |
| Myotoxin II     | Bothrops asper           | Monomeric; IA-sPLA\(_2\) (Asp49)                        | Myotoxicity; edema                              | 7600 (i.p.)                       | n.d.                             | None                                         | [41]      |
| CoaTX-II        | Crotalus oreganus abyssus | Dimeric; IA-sPLA\(_2\) (Asp49)                          | Myotoxicity; anticoagulant; edema               | 70 (i.c.v.)                       | n.d.                             | None                                         | [42]      |
| Cr-5            | Calloselasma rhodostoma  | Monomeric; IA-sPLA\(_2\) (Asp49)                        | Cytotoxicity; myotoxicity; edema                | 7000 (i.x.)                       | n.d.                             | None                                         | [44]      |
| Cr-IV 1         | Calloselasma rhadostoma  | Monomeric; IA-sPLA\(_2\) (Asp49)                        | Myotoxicity; cytotoxicity; edema                | 70 (i.c.v.)                       | n.d.                             | None                                         | [45]      |
| Ammodytoxin L   | Vipera ammodytes         | Monomeric; IA-sPLA\(_2\) (Ser49)                        | Myotoxicity                                    | 3600 (i.p.)                       | n.d.                             | None                                         | [46]      |
| Anticoagulant   |                          |                                                          |                                                 |                                  |                                  |                                             |           |
| Daboxin P       | Daboia russelli          | Monomeric; IA-sPLA\(_2\)                                | Strong anticoagulant                           | n.d.                             | FX; FXα                          | 1140                                         | [47]      |
| RVV-PHK\(^c\)   | D. russelli              | Monomeric; IA-sPLA\(_2\) (Asp49)                        | Anticoagulant                                  | 100 (i.p.)                        | n.d.                             | Yes                                          | [48]      |
| CM-IV           | Naja nigricollis        | Monomeric; IA-sPLA\(_2\) (Asp49)                        | Strongly anticoagulant; presynaptic neurotoxicity | 180 (i.p.)                        | FXα; FXII                        | Yes                                          | [49, 50]  |
| CM-II           | Naja mossambica         | Monomeric; IA-sPLA\(_2\)                                | Weak anticoagulant; myotoxicity; nephrotoxicity | n.d.                             | T\(_\text{F}^\text{SE}\) II          | Yes                                          | [51, 52]  |

\(^a\)BPTI, bovine pancreatic trypsin inhibitor; \(^b\)i.c.v, intracerebroventricular; \(i.v\), intravenous; \(i.c\), intracisternal; \(i.p\), intraperitoneal; n.d.: not determined; \(^c\)CaM, calmodulin; NP, neuronal pentraxin; PDI, protein disulide isomerase; TCBP-49, taipoxin-associated calcium-binding protein 49; M-sPLA\(_2\); R, M-type sPLA\(_2\); receptor. Fxa, blood coagulation factor Xα; FX, blood coagulation factor X; TF, tissue factor; FXII, blood coagulation factor VII; FVIIa, blood coagulation factor VIIα; v.d. K\(^+\) channel, voltage-dependent K\(^+\) channels; \(^d\)phospholipase A\(_2\) activity is in µmol/min/mg of toxin; Yes, original research paper does not show phospholipase A\(_2\) activity in concrete number or not in µmol/min/mg of toxin; None, all PLA\(_2\) homologues are here considered to be enzymatically inactive. Adapted from [50, 51].
as homodimers in solution connected by noncovalent bonds [56]. Previous studies focused on the fact that amino acids composition of synthetic peptides has revealed that the C-terminal regions of 115–129 residues, which are positively charged and full of basic, aromatic, hydrophobic residues, are the key structure in eliciting myotoxic effects [62, 63]. Site-directed mutagenesis experiments proved that Tyr117, Arg118, Tyr119, Lys122, and Phe125 also have significant impacts on the myotoxicity [64].

4.3. svPLA2 Anticoagulant Effect. The anticoagulant effect of svPLA2 usually causes bleeding in victim/prey by inhibiting one or two steps in the blood coagulation cascade. PLA2s can be classified as strong, weak, and nonanticoagulant based on the dose required to inhibit blood coagulation [65]. The hydrolysis of phospholipids by svPLA2 would be the primary mechanism to account for PLA2s' anticoagulation [66]. However, in the absence of phospholipids, some svPLA2s could also inhibit coagulation [67]. The correlation between svPLA2 enzymatic activity and anticoagulant effect is still unknown. Furthermore, there are other mechanisms that restrain coagulation, such as inhibition of the activation of the conversion of FX (blood coagulation factor X) to Fxa (blood coagulation factor Xa) and/or prothrombin to thrombin [68].

svPLA2s can also induce other toxic effects such as myoglobinuria-inducing, hemolytic, and platelet aggregation initiating/inhibiting activities [49]. Their wide distribution, conserved structures, and various severe pharmacological effects suggest that svPLA2s represent a promising target for new antivenom medicine. Indeed, there is sufficient evidence that PLA2 inhibitors (PLIs) are effective in using snake venom envenomation therapy [69].

5. PLA2 Inhibitors Attenuate Morbidity and Mortality of Snakebite Envenomation

Due to the high cost, long production period, limited categories, short storage life, and common clinical side-effects of current antivenin, scientists have attempted to create antioxidants from herbal extracts, marine compounds, mammalian and snake serum, and modified chemical molecules and peptides [70]. svPLA2s are the ideal target and widely used for antidote screening. Indeed, both natural and synthetic svPLA2 inhibitors are able to attenuate the morbidity and mortality of snakebite envenomation.

5.1. Natural svPLA2 Inhibitors from Plants, Marine Extracts, and Mammalian Serum. Medicinal plant extracts as traditional antivenoms have long been used in countries where the urotherapy is unobtainable [71]. In addition, these traditional and herbal treatments are often used as adjuvant therapies along with the antivenin treatment. Most plant antitoxic agents function by neutralizing svPLA2’s toxicity. An active glycoprotein (WSG) from Withania somnifera completely inhibits the cytotoxicity, edema, and myotoxicity of NN-Xia-PLA2 isolated from Naja atra venom, but fails to neutralize the neurotoxicity [72–74]. WSG has a similar structure to the α-chain of the PLIs derived from Australian elapid serum and was found to interact with NN-XIa–PLA2, but the mechanism currently remains unknown [74].

The aqueous extract of Casearia sylvestris was found to be effective against two snake venom toxins (Asp49-PLA2 and Lys49-PLA2 isolated from venom of B. moojeni, B. pirajai, B. neuwiedii, and B. jararacussu). Indeed, this plant has been found to inhibit myotoxicity, hemorrhage, anticoagulation, and edema [75, 76]. It is also able to prevent myonecrosis initiated by two Lys49-PLA2 toxins (PrTX-I from B. pirajai and BthTx-I from B. jararacussu venom) and neuromuscular blockages [77]. Recently research has shown that human secretory PLA2 inhibitors (e.g., quercetin, biflavonoid morelloflavone [78, 79]) isolated from plant extracts can also inhibit svPLA2.

Marine organisms are also a reservoir for antivenoms. Manoalide (MLD), a natural product from sponge Laffariella variabilis, can irreversibly inhibit extracellular PLA2 activity of cobra and rattlesnake venom with an IC50 value of 1.9 and 0.7 μM, respectively [80]. Its synthetic analogue, manoalogue (MLG), is also inhibitive to cobra PLA2 activity with an IC50 value of 7.5 μM [81].

Natural svPLA2 inhibitors also exist in some mammalian sera. DM64 is an acidic glycoprotein isolated from serum of the opossum, Didelphis marsupialis. DM64 can completely prevent myofiber breakdown caused by myotoxins I (Asp49) and II (Lys49) of B. asper venom [82]. N-glycosylation sites (Asn46, Asn179, Asn183, and Asn379) in this antimyotoxic protein play important roles in this inhibitory action [83].

5.2. Snake Blood PLA2 Inhibitors. Many venomous and nonvenomous snake species are naturally resistant to the deleterious actions of snake venom components. In many cases, this is due to the presence of specific antitoxins circulating in their blood. These alectorin factors are proteins generated in the snake's liver, with native molecular masses ranging from 75 to 180 kDa. These nonimmunoglobulin antitoxins are PLA2 inhibitors (i.e., snake blood phospholipase A2 inhibitors, sbPLIs) and are used to protect the snake from the internal or external envenomation.

sbPLIs can be produced by snakes of the Elapidae, Viperidae, Hydrophidae, Colubridae and Boidae families. These sbPLIs can be classified into three groups based on the homology of their amino acid sequence: α, β and γ [84]. Generally, the α and γ sbPLIs simultaneously occur in several snake species, while the βsbPLIs have only been reported in three snake species. When the target PLA2s are Lys49 homologues or Asp49 myotoxins, the sbPLIs are specifically called myotoxin inhibitor proteins (MIPs) [85, 86].

Since the first αPLI (BaMIP) was isolated from B. asper serum, 15 kinds of asbPLIs have been discovered in the different venomous snake families. Previous studies have shown that BaMIP can block both myotoxins I and III (isolated from B. asper venom) [87]. The αPLIs, αTIPLI, and αAbsPLI also show good inhibition of the enzymatic activities of acid-PLA2 (isolated from Viperidae). CgMIP-II and AnMIP can inhibit the basic-PLA2 enzymatic activities of Viperidae venom. BaMIP, BmjMIP and BjussuMIP can inhibit the enzymatic activities and toxic effects (i.e., edema, myotoxicity, and cytotoxicity) of acid/basic-PLA2. Furthermore, Quirós et al.
extracted a new myotoxin inhibitor αbPLI from A. nummifer serum (AmMIP) and found that this protein, at a ratio of 1:1, could decrease 67% of the A. nummifer myotoxin II and 93% of the B. asper myotoxin I [85].

Currently four kinds of βbPLIs have been found in three snake species. β PLI specifically inhibits the basic-PLA$_2$ enzymatic activities of Viperidae. The first βbPLI was purified from G. brevicaudus as a homotrimer and is specific for basic-PLA$_2$s from homologous venoms and forms a stable PLA$_2$-βbPLI complex at a molar ratio of 1:1 [88].

Twenty-three types of γbPLIs have been found in venomous and nonvenomous species. γ PLI from Elapidae and other nonvenomous snakes can inhibit PLA$_2$ activity in a range of different snake venoms. We recently reported a novel γ PLI isolated from the serum of Sinonatrix annularis, named γsaPLI, that showed a strong inhibition of lecithin degradation elicited by D. acutus venom PLA$_2$s in an in vitro study [89]. The γsaPLI was also effective in the inhibition of hemorrhagic toxicities elicited by D. acutus, N. atra, and A. halyx venoms [90].

5.3. Poly or Monoclonal Antibodies of svPLA$_2$ Are Effective in Neutralizing Snake Venom. Unlike the common antivenins of venom proteome, Garcia Denegri et al. developed a poly-antibody using a nontoxic PLA$_2$ (BaSpII RP4) from Bothrops alteratus as antigen [91]. This antibody showed a specific and sensitive inhibition of the venom PLA$_2$s’ enzymatic activity. Furthermore, the myotoxicity and mortality of the crude venom were significantly reduced in the presence of anti-PLA$_2$ IgG. When treated with a high dose of 2 × LD$_{50}$, equivalent to 112 µg of B. alternatus venom and 2.62 mg of IgG, all of the test animals survived after 48 h. In contrast, the control group (112 µg venom premuneculated with PBS) died within 4 hours. 5.25 mg of IgG treated animals could even endure as high as 4 times the LD$_{50}$ dose of venom (224 µg), with half of the treated group remaining alive at the end of 48 h. In contrast, the control group (224 µg venom premuneculated with PBS) died shortly within 90 mins.

Rodriguez et al. also produced an IgG against crotoxin (a basic PLA$_2$), the principle toxin of C. durissus terrificus (C.d.t.) with high myotoxicity and neurotoxic activities. Mice premuneculated with the anticrotoxin IgG showed low mortality after 24 and 48 h of inoculation (at 4 µg C.d.t. venom/test animal). The investigation showed that the IgGs of anti-PLA$_2$ were more effective than anticrotatic serum at neutralizing lethal activity [92]. Additionally, the anti-PLA$_2$ IgGs raised via immunization with P9a or P10a, two types of less toxic Cdt-PLA$_2$s, cross-reacted with all the isoforms of PLA$_2$s in the C.d.t. venom [93]. Although these antitoxic effects were only tested with their original venoms, the wide cross-reaction of these anti-PLA$_2$ IgGs with other svPLA$_2$s suggested that these compounds could likely also be used to neutralize other snake venoms. In other words, the improved neutralization activity of these anti-svPLA$_2$ IgGs indicates svPLA$_2$s are a promising target for broad-spectrum antivenom drug development.

5.4. Artificial Inhibitor of Mammal PLA$_2$ Exhibits Effective Antivenom Activity. Varespladib (LY315920) was designed as an inhibitor of the Ila, V, and X isoforms of the mammalian secretory phospholipase A$_2$ (sPLA$_2$). This compound acts as an anti-inflammatory agent by disrupting the first step of the arachidonic acid pathway of inflammation. From 2006 to 2012, varespladib was under active investigation by Anthera Pharmaceuticals for using as a potential therapy for several inflammatory diseases, including acute coronary syndrome and acute chest syndrome [94, 95]. Thought to be an effective antiatherosclerotic agent, varespladib showed promising therapeutic effects in reducing plasma sPLA$_2$ and low-density lipoprotein (LDL) [96].

Varespladib has recently been repurposed as an effective broad-spectrum svPLA$_2$ inhibitor and used for treatment of snakebite envenomation. Varespladib and its orally bioavailable prodrug methyl-varespladib (LY333013) showed strong inhibitory ability of 28 kinds of svPLA$_2$s from six continents. Indeed, the IC$_{50}$ values ranged from nano- to picomolars in an in vitro experiment [97]. Additionally, the compound elicited surprising effects with eastern coral snake (Micrurus fulvius) venom, which was considered to have the highest sPLA$_2$ activity and most intense hemo- and neurotoxic effects. Pretreatment with 0.1 mg of varespladib prolonged survival in mice at 4 times the LD$_{50}$ dose of eastern coral snake venom over the course of 8 h. All the negative control mice died at an average of 63 min, whereas the varespladib treatment group survived for an average of 1140 min. Varespladib also showed promising in vivo protection in Vipera berus envenomed mice. Mice treated with a subcutaneous injection of a 100% lethal dose of venom and varespladib survived for more than 24 h [97]. These findings are solid evidence of svPLA$_2$ being the target for a broad-spectrum antivenom.

6. Conclusions

svPLA$_2$s are widely distributed in snake venoms. A svPLA$_2$ could elicit one or more pharmacological effects (e.g., neurotoxicity, myotoxicity, anticoagulant, and edema). Furthermore, svPLA$_2$s can interact with other svPLA$_2$s (e.g., two different svPLA$_2$s, the “Asp” and “Lys” myotoxins from Bothrops asper, have been shown to synergistically enhance myonecrosis in in vitro and in vivo studies [98]) or other venom components (e.g., taicatoxin, a Ca$^{2+}$ channel inhibitor composed of an α-neurotoxin-like peptide, a neurotoxic phospholipase A$_2$, and a serine protease inhibitor, connected by noncovalent bonds [99]).

A variety of PLA$_2$ inhibitors were discovered or synthesized in the past few decades. Most inhibitors extracted from medical plants, marine animals, and mammalian serum specially inhibit svPLA$_2$ toxicity. sbPLIs are natural, endogenous protective components against snake venom, among which the γ PLI were commonly inhibitory to different category of venom [100]. Anti-PLA$_2$ antibodies could specifically inactivate enzymatic activity and toxicity, both with the original venom and other svPLA$_2$s [93]. Indeed, some of these compounds could function even better than the antivenin that is currently clinically applied [92]. A synthetic human sPLA$_2$ inhibitor varespladib was found to possess the ability to neutralize a variety of snake venoms.
worldwide, with significant prolongation of survival time on rats that were inoculated with varespladib simultaneously or following exposure [97]. In conclusion, the anti-PLA$_2$ drugs are promising antitoxins for a broad-spectrum of snake venoms and other animal toxins and could also be effective in prevention of inflammatory reactions (i.e., systemic toxicological syndromes).

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

**Authors’ Contributions**
Huixiang Xiao and Hong Pan contributed equally to this work and are considered as co-first authors.

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**References**
[1] D. A. Warrell, “Snake bite,” *The Lancet*, vol. 375, no. 9708, pp. 77–88, 2010.
[2] J.-P. Chippaux, “Snake-bites: appraisal of the global situation,” *Bulletin of the World Health Organization*, vol. 76, no. 5, pp. 515–524, 1998.
[3] Editorial, “Snake bite—the neglected tropical disease,” *Lancet*, vol. 386, no. 9999, pp. 1100, 2015.
[4] J. M. Gutiérrez, R. D. G. Theakston, and D. A. Warrell, “Confronting the neglected problem of snake bite envenoming: the need for a global partnership,” *PLoS Medicine*, vol. 3, no. 6, pp. 0727–0731, 2006.
[5] H. A. De Silva, N. M. Ryan, and H. J. De Silva, “Adverse reactions to snake antivenom, and their prevention and treatment,” *British Journal of Clinical Pharmacology*, vol. 81, no. 3, pp. 446–452, 2016.
[6] J. M. Gutiérrez, D. Williams, H. W. Fan, and D. A. Warrell, “Snakebite envenoming from a global perspective: towards an integrated approach,” *Toxicon*, vol. 56, no. 7, pp. 1223–1235, 2010.
[7] Q. Schiermeier, “Africa braced for snakebite crisis,” *Nature*, vol. 525, no. 7569, p. 299, 2015.
[8] D. L. Scott, “Phospholipase A$_2$ structure and catalytic properties,” in *In Venom Phospholipase A$_2$ Enzymes: Structure, Function, and Mechanism*, R. M. Kini, Ed., pp. 97–128, John Wiley, Chichester, UK, 1997.
[9] E. A. Dennis, J. Cao, Y.-H. Hsu, V. Magrioti, and G. Kokotos, “Phospholipase A$_2$ enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention,” *Chemical Reviews*, vol. 111, no. 10, pp. 6130–6185, 2011.
[10] R. H. Schaloske and E. A. Dennis, “The phospholipase A$_2$ superfamily and its group numbering system,” *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, vol. 1761, no. 11, pp. 1246–1259, 2006.
[11] D. A. Six and E. A. Dennis, “The expanding superfamily of phospholipase A2 enzymes: classification and characterization,” *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, vol. 1488, no. 1–2, pp. 1–19, 2000.
[12] J. Fohlman, P. Lind, and D. Eaker, “Taipoxin, an extremely potent presynaptic snake venom neurotoxin Elucidation of the primary structure of the acidic carbohydrate-containing taipoxin-subunit, a pro phospholipase homolog,” *FEBS Letters*, vol. 84, no. 2, pp. 367–371, 1977.
[13] J. A. Pearson, M. I. Tyler, K. V. Retson, and M. E. H. Howden, “Studies on the subunit structure of textilotoxin, a potent presynaptic neurotoxin from the venom of the Australian common brown snake (Pseudonaja textilis). 3. The complete amino-acid sequences of all the subunits,” *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, vol. 1161, no. 2–3, pp. 223–229, 1993.
[14] B. R. Francis, N. Jorge Da Silva Jr., C. Seebart, L. L. Casais E Silva, J. J. Schmidt, and I. I. Kaiser, “Toxins isolated from the venom of the Brazilian coral snake (Micrurus frontalis frontalis) include hemorrhagic type phospholipases A2 and postsynaptic neurotoxins,” *Toxicon*, vol. 35, no. 8, pp. 1193–1203, 1997.
[15] R. C. De Paula, H. C. Castro, C. R. Rodrigues, P. A. Melo, and A. L. Fuly, “Structural and pharmacological features of phospholipases A$_2$ from snake venoms,” *Protein and Peptide Letters*, vol. 16, no. 8, pp. 899–907, 2009.
[16] S. P. Mackessy, “Snake Venom Phospholipase A$_2$ Enzymes,” in *Handbook of Venoms and Toxins of Reptiles*, S. P. Mackessy, Ed., pp. 174–195, Taylor and Francis, Boca Raton, Fla, USA, 2010.
[17] T. Petan, I. Krizaj, and J. Pungercar, “Restoration of enzymatic activity in a Ser-49 phospholipase A$_2$ homologue decreases its Ca$^{2+}$-independent membrane-damaging activity and increases its toxicity,” *Biochemistry*, vol. 46, no. 44, pp. 12795–12809, 2007.
[18] R. J. Ward, L. Chioato, A. H. C. De Oliveira, R. Ruller, and J. M. Sá, “Active-site mutagenesis of a Lys49-phospholipase A$_2$: Biological and membrane-disrupting activities in the absence of catalysis,” *Biochemical Journal*, vol. 362, no. 1, pp. 89–96, 2002.
[19] C. Bon, “Multicomponent neurotoxic phospholipases A$_2$,” in *Venom Phospholipase A$_2$ Enzymes: Structure, Function, and Mechanism*, R. M. Kini, Ed., pp. 269–285, John Wiley, Chichester, UK, 1997.
[20] S. P. Mackessy, “The field of reptile toxinology snakes, lizards, and their venoms,” in *In Handbook of Venoms and Toxins of Reptiles*, S. P. Mackessy, Ed., pp. 3–19, Taylor and Francis, Boca Raton, Fla, USA, 2010.
[21] J. J. Calvete, “Proteomics in venom research: a focus on PLA$_2$ molecules,” *Acta Chimica Slovenica*, vol. 58, no. 4, pp. 629–637, 2011.
[22] D. Georgieva, R. K. Arni, and C. Betzel, “Proteome analysis of snake venom toxins: pharmacological insights,” *Expert Review of Proteomics*, vol. 5, no. 6, pp. 787–797, 2008.
[23] R. H. Ziganshin, S. I. Kovalchuk, G. P. Arapidi et al., “Quantitative proteomic analysis of vietnamese krait venoms: neurotoxins are the major components in bungarus multicinctus and phospholipases A$_2$ in bungarus fasciatus,” *Toxicon*, vol. 107, pp. 197–209, 2015.
[24] J. M. Gutiérrez and B. Lomonte, “Phospholipases A$_2$: unveiling the secrets of a functionally versatile group of snake venom toxins,” *Toxicon*, vol. 62, pp. 27–39, 2013.
[25] R. M. Kini, “Excitement ahead: structure, function and mechanism of snake venom phospholipase A$_2$ enzymes,” *Toxicon*, vol. 42, no. 8, pp. 827–840, 2003.
[26] S. C. Sampaio, S. Hyslop, M. R. M. Fontes et al., “Crototoxin: novel activities for a classical β-neurotoxin,” *Toxicon*, vol. 55, no. 6, pp. 1045–1060, 2010.

[27] A. L. C. Terra, L. S. Moreira-Dill, R. Simões-Silva et al., “Biological characterization of the amazon coral micrurus spixii snake venom: isolation of a new neurotoxic phospholipase A₂,” *Toxicon*, vol. 103, pp. 1–11, 2015.

[28] G. Lambeau, P. Ancian, J. Barhanin, and M. Lazdunski, “Cloning and expression of a membrane receptor for secretory phospholipases A₂,” *The Journal of Biological Chemistry*, vol. 269, no. 3, pp. 1575–1578, 1994.

[29] G. Lambeau, A. Schmid-Alliana, M. Lazdunski, and J. Barhanin, “Identification and purification of a very high affinity binding protein for toxic phospholipases A₂ in skeletal muscle,” *The Journal of Biological Chemistry*, vol. 265, no. 16, pp. 9526–9532, 1990.

[30] B. V. Lipps, “Isolation of subunits, α, β and γ of the complex taipoxin from the venom of Australian taipain snake (Oxyuranus s. scutellatus): characterization of β taipoxin as a potent mitogen,” *Toxicon*, vol. 38, no. 12, pp. 1845–1854, 2000.

[31] A. Coulter, R. Harris, A. Broad et al., “The isolation and some properties of the major neurotoxic component from the venom of the common or Eastern Australian brown snake (Pseudonaja textilis),” *Toxicon*, vol. 21, no. 3, pp. 81–84, 1983.

[32] G. Faure, V. T. Gowda, and R. C. Maroun, “Characterization of a human coagulation factor Xa-binding site on Viperidae snake venom phospholipases A₂ by affinity binding studies and molecular bioinformatics,” *BMC Structural Biology*, vol. 7, article no. 82, 2007.

[33] J. Šribar, A. Copić, A. Pariš et al., “A high affinity acceptor for phospholipase A₂ with neurotoxic activity is a calmodulin,” *The Journal of Biological Chemistry*, vol. 276, no. 16, pp. 12493–12496, 2001.

[34] J. Šribar, N. E. Sherman, P. Prijatelj et al., “The neurotoxic phospholipase A₂ associates, through a non-phosphorylated binding motif, with 14-3-3 protein γ and ε isoforms,” *Biochemical and Biophysical Research Communications*, vol. 302, no. 4, pp. 691–696, 2003.

[35] N. Vardjan, N. E. Sherman, J. Pungerčar, J. W. Fox, F. Gubensek, and I. Križaj, “High-molecular-mass receptors for ammodytin in pig are tissue-specific isoforms of M-type phospholipase A₂ receptor,” *Biochemical and Biophysical Research Communications*, vol. 289, no. 1, pp. 143–149, 2001.

[36] K. Kondo, H. Toda, K. Narita, and C.-Y. Lee, “Amino acid sequences of three β-bungarotoxins (β3-, β4-, and β5-bungarotoxins) from Bungarus multicinctus Venom. amino acid substitutions in the A chains,” *The Journal of Biochemistry*, vol. 91, no. 5, pp. 1531–1548, 1982.

[37] M. J. Sutcliffe, C. M. Dobson, and R. E. Oswald, “Solution structure of neuronal bungarotoxin determined by two-dimensional NMR spectroscopy: calculation of tertiary structure using systematic homologous model building, dynamical simulated annealing, and restrained molecular dynamics,” *Biochemistry*, vol. 31, no. 11, pp. 2962–2970, 1992.

[38] J. Halpert and D. Eaker, “Amino acid sequence of a presynaptic neurotoxin from the venom of Notechis scutatus scutatus (Australian tiger snake),” *The Journal of Biological Chemistry*, vol. 250, no. 17, pp. 6990–6997, 1975.

[39] B. Westerlund, P. Nordlund, U. Uhlin, D. Eaker, and H. Eklund, “The three-dimensional structure of notexin, a presynaptic neurotoxic phospholipase A₂ at 2.0 Å resolution,” *FEBS Letters*, vol. 301, no. 2, pp. 159–164, 1992.

[40] I. I. Kaiser, J. M. Gutierrez, D. Plummer, S. D. Aird, and G. V. Odell, “The amino acid sequence of a myotoxic phospholipase from the venom of Bothrops asper,” *Archives of Biochemistry and Biophysics*, vol. 278, no. 2, pp. 319–325, 1990.

[41] A. M. Soares, V. M. Rodrigues, M. I. Homsi-Brandeburgo et al., “A rapid procedure for the isolation of the LYS-49 myotoxin II from bothrops moojeni (caissaca) venom: biochemical characterization, crystallization, myotoxic and edematogenic activity,” *Toxicon*, vol. 36, no. 3, pp. 503–514, 1998.

[42] J. R. Almeida, M. Lancellotti, A. M. Soares et al., “CoxaTx-II, a new dimeric Lys49 phospholipase A₂ from Crotalus oreganus abyssus snake venom with bactericidal potential: Insights into its structure and biological roles,” *Toxicon*, vol. 120, pp. 147–158, 2016.

[43] V. L. Bonfim, L. A. Ponce-Soto, J. C. Novello, and S. Marangoni, “Structural and functional properties of Cr5, a new Lys49 phospholipase A₂ homologue isolated from the venom of the snake Calloselasma rhodostoma,” *The Protein Journal*, vol. 25, no. 7-8, pp. 492–502, 2006.

[44] L. A. Ponce-Soto, B. Lomonte, J. M. Gutiérrez, L. Rodrigues-Simioni, J. C. Novello, and S. Marangoni, “Structural and functional properties of BaTX, a new Lys49 phospholipase A₂ homologue isolated from the venom of the snake Bothrops alternatus,” *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 1770, no. 4, pp. 585–593, 2007.

[45] V. L. Bonfim, L. A. Ponce-Soto, D. Martins de Souza et al., “Structural and functional characterization of myotoxin, CrIV 1, a phospholipase A₂, D49 from the venom of the snake Calloselasma rhodostoma,” *Biologicals*, vol. 36, no. 3, pp. 168–176, 2008.

[46] I. KRIŽAJ, A. L. BIEBER, A. RITONJA, and F. GUBENSEK, “The primary structure of ammodytin L, a myotoxic phospholipase A₂ homologue from Vipera ammodytes venom,” *European Journal of Biochemistry*, vol. 202, no. 3, pp. 1165–1168, 1991.

[47] M. Sharma, J. K. Iyer, N. Shih et al., “Daboxin p, a major phospholipase A₂ enzyme from the Indian daboia russelli russelli venom targets factor x and factor xa for its anticoagulant activity,” *PLoS ONE*, vol. 11, no. 4, Article ID e0153770, 2016.

[48] A. K. Chakraborty, R. H. Hall, and A. C. Ghose, “Purification and characterization of a potent hemolytic toxin with phospholipase A₂ activity from the venom of Indian Russell’s viper,” *Molecular and Cellular Biochemistry*, vol. 237, no. 1-2, pp. 95–102, 2002.

[49] R. T. Kerns, R. M. Kini, S. Stefansson, and H. J. Evans, “Targeting of venom phospholipases: The strongly anticoagulant phospholipase A₁ from Naja nigricollis venom binds to coagulation factor Xa to inhibit the prothrombinase complex,” *Archives of Biochemistry and Biophysics*, vol. 369, no. 1, pp. 107–113, 1999.

[50] R. M. Kini, “Structure-function relationships and mechanism of anticoagulant phospholipase A₂ enzymes from snake venoms,” *Toxicon*, vol. 45, no. 8, pp. 1147–1161, 2005.

[51] F. J. Joubert, “Naja mossambica mossambica venom Purification, some properties and the amino acid sequences of three phospholipases A (CM-I, CM-II and CM-III),” *BBA - Protein Structure*, vol. 493, no. 1, pp. 216–227, 1977.

[52] W. W. Lin, P. L. Chang, C. Y. Lee, and F. J. Joubert, “Pharmacological study on phospholipases A₂ isolated from Naja mossambica mossambica venom,” *Proceedings of the National Science Council, Republic of China, Part B, Life Sciences*, vol. 11, no. 2, pp. 155–163, 1987.

[53] U. K. Ranawaka, D. G. Laloo, H. J. de Silva, and J. White, “Neurotoxicity in snakebite—the limits of our knowledge,”
PLOS Neglected Tropical Diseases, vol. 7, no. 10, Article ID e2302, 2013.

[54] J. Pungerčar and I. Križaj, “Understanding the molecular mechanism underlying the presynaptic toxicity of secreted phospholipases A₂,” Toxicon, vol. 50, no. 7, pp. 871–892, 2007.

[55] T. Petan, I. Križaj, M. H. Gelb, and J. Pungerčar, “Ammodonytoxins, potent presynaptic neurotoxins, are also highly efficient phospholipase A₂ enzymes,” Biochemistry, vol. 44, no. 37, pp. 12535–12545, 2005.

[56] B. Lomonte and J. Rangel, “Snake venom Lys49 myotoxins: from phospholipases A₂ to non-enzymatic membrane disruptors,” Toxicon, vol. 60, no. 4, pp. 520–530, 2012.

[57] R. Otero, J. Gutiérrez, M. Beatriz Mesa et al., “Complications of Bothrops, Porthidium, and Bothriechis snakebites in Colombia. A clinical and epidemiological study of 39 cases attended in a university hospital,” Toxicon, vol. 40, no. 8, pp. 1107–1114, 2002.

[58] J. M. Gutiérrez and C. L. Ownby, “Skeletal muscle degeneration induced by venom phospholipases A₂: insights into the mechanisms of local and systemic myotoxicity,” Toxicon, vol. 42, no. 8, pp. 915–931, 2003.

[59] B. Lomonte and J. M. Gutiérrez, “Phospholipases A₂ from vipers: how do they induce skeletal muscle damage?” Acta Chimica Slovenica, vol. 58, no. 4, pp. 647–659, 2011.

[60] B. Lomonte, E. Moreno, A. Tarkowski, L. Á. Hanson, and M. Maccarana, “Neutralization interaction between heparin and myotoxin II, a lysine 49 phospholipase A₂ from Bothrops asper snake venom: identification of a heparin-binding and cytolytic toxin region by the use of synthetic peptides and molecular modeling,” The Journal of Biological Chemistry, vol. 269, no. 47, pp. 29867–29873, 1994.

[61] C. E. Núez, Y. Angulo, and B. Lomonte, “Identification of the myotoxic site of the Lys49 phospholipase A₂ from Agkistrodon piscivorus piscivorus snake venom: Synthetic C-terminal peptides from Lys49, but not from Asp49 myotoxins, exert membrane-damaging activities,” Toxicon, vol. 39, no. 10, pp. 1587–1594, 2001.

[62] L. Chioato, E. A. Aragão, T. Lopes Ferreira, A. Ivo de Medeiros, L. H. Faccioli, and R. J. Ward, “Mapping of the structural determinants of artificial and biological membrane damaging activities of a Lys49 phospholipase A₂ by scanning alanine mutagenesis,” Biochimica et Biophysica Acta (BBA) - Biomembranes, vol. 1768, no. 5, pp. 1247–1257, 2007.

[63] H. M. Verheij, M.-C. Boffa, C. Rothen, M. Bryckaert, R. Verger, and G. H. de Haas, “Correlation of Enzymatic Activity and Anticoagulant Properties of Phospholipase A₂,” European Journal of Biochemistry, vol. 112, no. 1, pp. 25–32, 1980.

[64] R. M. Kini, “Anticoagulant proteins from snake venoms: Structure, function and mechanism,” Biochemical Journal, vol. 397, no. 3, pp. 377–387, 2006.

[65] D. Saikia, R. Thakur, and A. K. Mukherjee, “An acidic phospholipase A₂ (RVVA-PLA2-I) purified from Daboia russelli venom exerts its anticoagulant activity by enzymatic hydrolysis of plasma phospholipids and by non-enzymatic inhibition of factor Xa in a phospholipid/Ca²⁺ independent manner,” Toxicon, vol. 57, no. 6, pp. 841–850, 2011.

[66] S. Stefansson, R. M. Kini, and H. J. Evans, “The basic phospholipase A₂ from Naja nigricollis venom inhibits the prothrombinase complex by a novel nonenzymatic mechanism,” Biochemistry, vol. 29, no. 33, pp. 7742–7746, 1990.

[67] R. P. Samy, P. Gopalakrishnakone, and V. T. Chow, “Therapeutic application of natural inhibitors against snake venom phospholipase A₂,” Bioinformation, vol. 8, no. 1, pp. 48–57, 2012.

[68] S. Marcucci, C. D. Sant’Ana, C. Z. Oliveira et al., “Snake venom phospholipase A₂ inhibitors: Medicinal chemistry and therapeutic potential,” Current Topics in Medicinal Chemistry, vol. 7, no. 8, pp. 743–756, 2007.

[69] A. M. Soares, F. K. Ticli, S. Marcucci et al., “Medicinal plants with inhibitory properties against snake venoms,” Current Medicinal Chemistry, vol. 12, no. 22, pp. 2625–2641, 2005.

[70] M. Deepa and T. Veerabasappa Gowda, “Purification and characterization of a glycoprotein inhibitor of toxic phospholipase from Withania somnifera,” Archives of Biochemistry and Biophysics, vol. 408, no. 1, pp. 42–50, 2002.

[71] L. Mishra, B. B. Singh, and S. Dagenais, “Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review,” Alternative Medicine Review, vol. 5, no. 4, pp. 334–346, 2000.

[72] D. K. Machiah and T. V. Gowda, “Purification of a post-synaptic neurotoxic phospholipase A₂ from Naja naja venom and its inhibition by a glycoprotein from Withania somnifera,” Biochimie, vol. 88, no. 6, pp. 701–710, 2006.

[73] M. H. Borges, A. M. Soares, V. M. Rodrigues et al., “Effects of aqueous extract of Casearia sylvestris (Flacourtiaceae) on actions of snake and bee venoms and on activity of phospholipases A₂,” Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology, vol. 127, no. 1, pp. 21–30, 2000.

[74] M. H. Borges, A. M. Soares, V. M. Rodrigues et al., “Neutralization of proteases from Bothrops snake venoms by the aqueous extract from Casearia sylvestris (Flacourtiaceae),” Toxicon, vol. 39, no. 12, pp. 1863–1869, 2001.

[75] W. L. G. Cavalcante, T. O. Campos, M. Dal Pai-Silva et al., “Neutralization of snake venom phospholipase A₂ toxins by aqueous extract of Casearia sylvestris (Flacourtiaceae) in mouse neuromuscular preparation,” Journal of Ethnopharmacology, vol. 112, no. 3, pp. 490–497, 2007.

[76] J. A. Perean˜ez, A. C. Patiño, V. Núñez, and E. Osorio, “The bilavonoid morelloflavone inhibits the enzymatic and biological activities of a snake venom phospholipase A₂,” Chemical-Biological Interactions, vol. 220, pp. 94–101, 2014.

[77] C. A. Cotrim, S. C. B. De Oliveira, E. B. S. Diz Filho et al., “Quercetin as an inhibitor of snake venom secretory phospholipase A₂,” Chemico-Biological Interactions, vol. 189, no. 1-2, pp. 9–16, 2011.

[78] C. F. Bennett, S. Mong, M. A. Clarke, L. I. Kruse, and S. T. Crooke, “Differential effects of manoolide on secreted and intracellular phospholipases,” Biochemical Pharmacology, vol. 36, no. 5, pp. 733–740, 1987.

[79] L. J. Reynolds, B. P. Morgan, G. A. Hite, E. D. Mihelich, and E. A. Dennis, “Phospholipase A₂ inhibition and modification by manoolide,” Journal of the American Chemical Society, vol. 110, no. 15, pp. 5172–5177, 1988.
[82] S. L. G. Rocha, B. Lomonte, A. G. C. Neves-Ferreira et al., “Functional analysis of DM64, an antinmyotoxic protein with immunoglobulin-like structure from Dendrophis maruaplalisk serum,” *European Journal of Biochemistry*, vol. 269, no. 24, pp. 6052–6062, 2002.

[83] I. R. León, A. G. da Costa Neves-Ferreira, S. L. G. da Rocha, M. R. de Oliveira Trujillo, J. Perales, and R. H. Valente, “Using mass spectrometry to explore the neglected glycan moieties of the antiophidian proteins DM43 and DM64,” *Proteomics*, vol. 12, no. 17, pp. 2753–2765, 2012.

[84] S. Lizano, G. Domont, and J. Perales, “Natural phospholipase A₂ myotoxin inhibitor proteins from snakes, mammals and plants,” *Toxicon*, vol. 42, no. 8, pp. 963–977, 2003.

[85] S. Quirós, A. Alape-Girón, Y. Angulo, and B. Lomonte, “Isolation, characterization and molecular cloning of AnMIP, a new α-type phospholipase A₂ myotoxin inhibitor from the plasma of the snake Atropoides nummifer (Viperidae: Crotalinae),” *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, vol. 146, no. 1, pp. 60–68, 2007.

[86] C. Z. Oliveira, N. A. Santos-Filho, D. L. Menaldo et al., “Structural and functional characterization of a γ-type phospholipase A₂ inhibitor from Bothrops jararacussu Snake Plasma,” *Current Topics in Medicinal Chemistry*, vol. 11, no. 20, pp. 2509–2519, 2011.

[87] S. Lizano, B. Lomonte, J. W. Fox, and J. M. Gutiérrez, “Biochemical characterization and pharmacological properties of a phospholipase A₂ myotoxin inhibitor from the plasma of the snake Bothrops asper,” *Biochemical Journal*, vol. 326, no. 3, pp. 853–859, 1997.

[88] N. Ohsuka, H. Okuhara, S. Inoue, K. Ikeda, and K. Hayashi, “Purification and characterization of three distinct types of phospholipase A₂ inhibitors from the blood plasma of the Chinese mamushi, Agkistrodon blomhoffii sinicus,” *Biochemical Journal*, vol. 325, no. 2, pp. 527–531, 1997.

[89] K. Chen, L.-P. Zhong, L.-Z. Chen, X. Li, X. Xu, and C.-H. Huang, “Investigation and purification of snake venom secretory phospholipase A₂ inhibitors from sera of some common snake species in Jiangxi province,” *Pharmaceutical Biotechnology*, vol. 18, no. 3, pp. 220–223, 2011.

[90] Z. Le, X. Li, P. Yuan, P. Liu, and C. Huang, “Orthogonal optimization of prokaryotic expression of a natural snake venom phospholipase A₂ inhibitor from Sinonatrix annularis,” *Toxicon*, vol. 108, pp. 264–271, 2015.

[91] M. E. García Denegri, S. Maruñak, J. S. Todaro, L. A. Ponce-Soto, O. Acosta, and L. Leiva, “Neutralisation of the pharmacological activities of Bothrops alternatus venom by anti-PLA₂ IgGs,” *Toxicon*, vol. 86, pp. 89–95, 2014.

[92] J. P. Rodriguez, M. De Marzi, S. Maruñak, E. L. Malchiodi, L. C. Leiva, and O. Acosta, “Rabbit IgG antibodies against phospholipase A₂ from Crotalus durissus terrificus neutralize the lethal activity of the venom,” *Medicina*, vol. 66, no. 6, pp. 512–516, 2006.

[93] L. S. Fusco, J. P. Rodriguez, F. Torres-Huaco et al., “P9a(CdtPLA₂) from Crotalus durissus terrificus as good immunogen to be employed in the production of croatalic anti-PLA₂ IgG,” *Toxicology Letters*, vol. 238, no. 1, pp. 7–16, 2015.

[94] M. Karakas and W. Koenig, “Varespladib methyl, an oral phospholipase A₂ inhibitor for the potential treatment of coronary artery disease,” *IDrugs*, vol. 12, no. 9, pp. 585–592, 2009.