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REVIEW ARTICLE

Pharmacology of spider venom toxins

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Abstract

Spider venom is an intricate combination of target specific enzymatic and non-enzymatic toxins. In addition, the venom also contains polyamine neurotoxins, ATP, AMP, ADP, guanosine, 2,4,6-trihydroxy purine, γ-aminobutyric acid, glutamic acid, aspartic acid, taurine, histamine, serotonin, tyramine, octomine, nor-adrenaline and inorganic salts. Several enzymes such as hyaluronidase, protease, phospholipase D, sphingomyelinase and neurotoxic peptides have been extensively characterized from spider venoms. Spider bite is an accidental event; envenomation can cause both local (edema, hemorrhage, myo/dermonecrosis) and systemic toxicity (neurotoxicity, myotoxicity, cytotoxicity and hemostatic alterations). While, the latter is pertaining to the very few groups of spiders, namely, *Loxosceles species and Hippasa partita*. The local and systemic toxicity may be attributed to the synergistic effect of both enzymatic and non-enzymatic toxins. More importantly, spider venom components possess immense potential for biotechnological and therapeutic applications. In addition, they have also been used as prototypes in drug design. Based on these facts, this review makes an attempt to provide an insight into the pharmacology of enzymatic toxins (Sphingomyelinase, Hyaluronidase, Phospholipase, Protease, Collagenase, Phosphodiesterase, ATPases, Alkaline phosphatase and Peptide isomerases) and non-enzymatic toxins (translationally controlled tumor proteins and serine protease inhibitors).

Keywords

Enzymatic, local and systematic toxicity, non-enzymatic toxins, spider venom

History

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Introduction

Spiders are diversified group of arthropods. Nearly 50 000 species of approximately 112 families have been identified (Platnick, 2014). The majorities of spider bites are accidental and cause only minor effects (Isbister & Gray, 2002). In contrast, spiders of the genera *Atrax, Loxosceles, Lactrodectus, Phoneutria, Lithyphantus, Sicarius* and *Hadronyche* were reported to cause lethal effects (Habermehl, 1984; Isbister & White 2004). Spider venom is a complex cocktail of principally enzymatic and non-enzymatic protein and peptide toxins (Figure 1). In addition, polyamine neurotoxins, free amino acids, monoamines and inorganic salts are also present (Baron et al., 2013; Gremski et al., 2014; Jackson & Parks, 1989; Min et al., 2013; Vassilevski & Grishin, 2011). The key function of the venom is prey acquisition and defense. Spider venom has been found to cause a wide range of pharmacological effects, including edema, hemorrhage, dermo/myonecrosis, hemolysis, inflammation, neurotoxicity, cytotoxicity, alteration in coagulation and platelet function (Devaraja et al., 2008, 2010, 2011; Nagaraju et al., 2006; Rash & Hodgson, 2002). Several neurotoxic, cytotoxic, antimicrobial, anti-insecticidal, anti-arrhythmic, antiparasitic, trypsin inhibitory peptides and enzymes such as hyaluronidase, proteases, phospholipase D and sphingomyelinase D have been isolated and characterized from various spider venoms (Davletov et al., 2012; Isbister & Gray, 2002; King & Hardy, 2013; Nimmrich & Gross, 2012; Windley et al., 2012). Spider venom peptide toxins have been extensively studied through proteomic and transcriptomic approaches (He et al., 2013; Klint et al., 2012). Recently, spider venom toxins have been broadly used for novel therapeutic/biotechnological applications and they have been widely reviewed (Baron et al., 2013; Davletov et al., 2012; Gremski et al., 2014; King et al., 2013; Klint et al., 2012; Min et al., 2013; Nimmrich et al., 2012; Nunes et al., 2013; Windley et al., 2012). This review specifically provides an insight into the pharmacology of enzymatic toxins (Sphingomyelinase, Hyaluronidase, Phospholipase, Protease, Collagenase, Phosphodiesterase, ATPases, Alkaline phosphatase and Peptide isomerases) and non-enzymatic toxins (translationally controlled tumor proteins and serine protease inhibitors).

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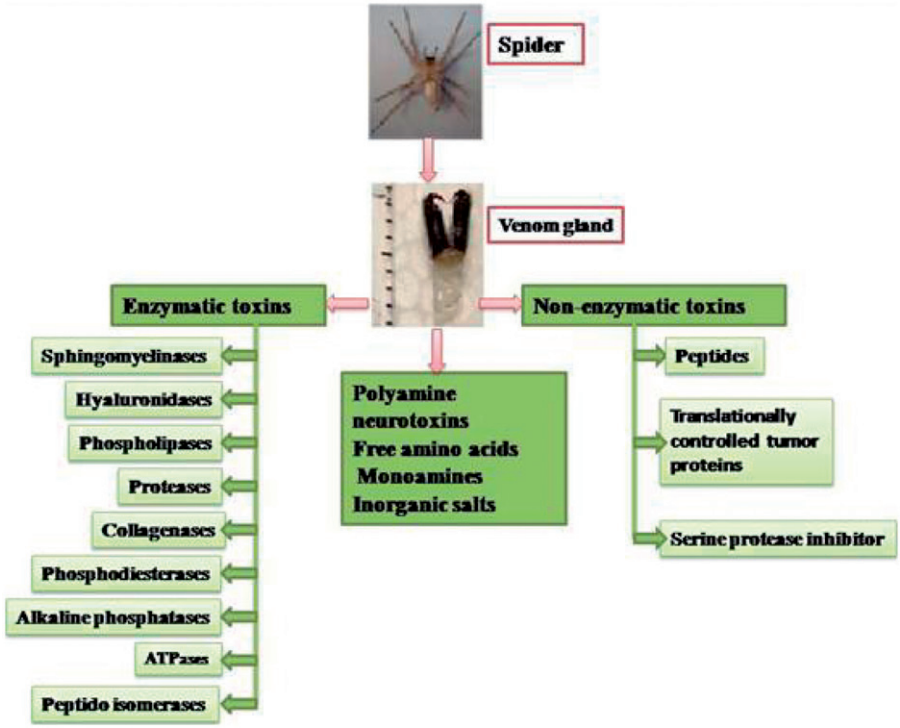
Figure 1. Major constituents of spider venom.



Enzymatic toxins

Sphingomyelinases

Sphingomyelinases are the most comprehensively studied enzymes of spider venom. They degrade sphingomyelin to ceramide, which is a known mediator of apoptosis. There are a number of sphingomyelinases with differing pH optima that appear to operate in different regions of the cell with potentially distinct biochemical roles. In venoms, sphingomyelinase-D is known to contribute to the major toxic effect and induce red blood cell lysis, platelet aggregation, heart dysfunction and dermonecrotic lesions (Binford et al., 2005; de Santi Ferrara et al., 2009; Dias-Lopes et al., 2010, 2014; Forrester et al., 1978; Futrell, 1992; Kurpiewski et al., 1981; Stock et al., 2012; Tambourgi et al., 1998; Tavares et al., 2011; Zobel-Thropp et al., 2010). Figure 2 represents the pharmacological properties of sphingomyelinase. Sphingomyelinase D belongs to a gene family of multiple venom-expressed members that vary in functional specificity (Greta et al., 2005). Different isoforms of sphingomyelinase D with molecular mass range of 31–35 kDa were identified in the venoms of *Loxosceles intermedia, recluse, gaucho* and *laeta* (Sánchez-Olivas et al., 2011). The pharmacology of spingomyelinase is influenced by the varied geographical distribution of the spiders (Zobel-Thropp et al., 2010). According to recent findings, the sphingomylinase from *Loxosceles* species can stimulate production of interleukin-8 and granulocyte-macrophage colony-stimulating factor, which in turn causes exanthematous pustulosis Coombs-positive hemolytic anemia in victims (Lane et al., 2011). Zobel-Thropp et al. (2012) reported that recombinant sphingomyelinase D (Laz-SMase D) is a potent insecticidal toxin. Stock et al. (2012) showed that ceramide-1-phosphate is a degradative end product of sphingomyelinase D and it was found to alter the targeted membranes structure. Lopes et al. (2013)

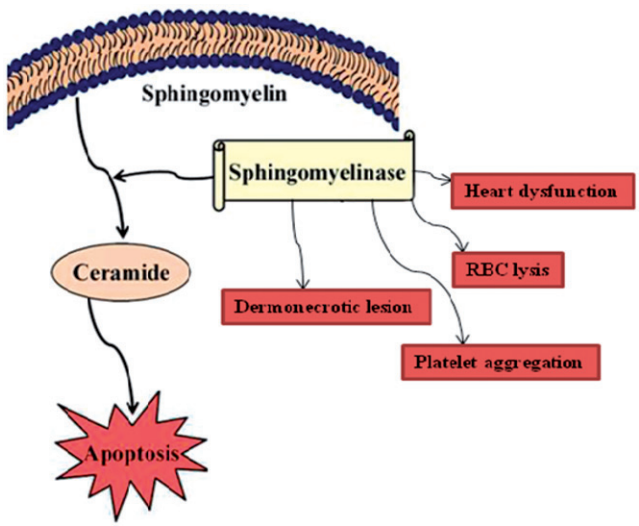


Figure 2. Pharmacological properties of sphingomyelinase.

reported that sphingomyelinase D from the Brazilian spider *Sicarius ornatus* causes haemolysis and keratinocyte cell death. Spider-venom sphingomyelinase D is a potent activator of an endogenous metalloprotease that can specifically hydrolyze the C5a receptor (C5a R) that in turn cause membrane asymmetry and hemolysis (Dias-Lopes et al., 2014; Van den Berg et al., 2012).

Hyaluronidases

Hyaluronidases are endo-β-glycosidases that hydrolyze hyaluronic acid and chondroitin sulphate (Manzel & Farr 1988; da Silveira et al., 2007a,b,c). Kaiser (1956) was the first to report hyaluronidase activity from the venom of the Brazilian spiders *Lycosa raptorial* and *Ctenus nigriventer* (now *Phoneutria nigriventer*). Schanbacher et al. (1973)

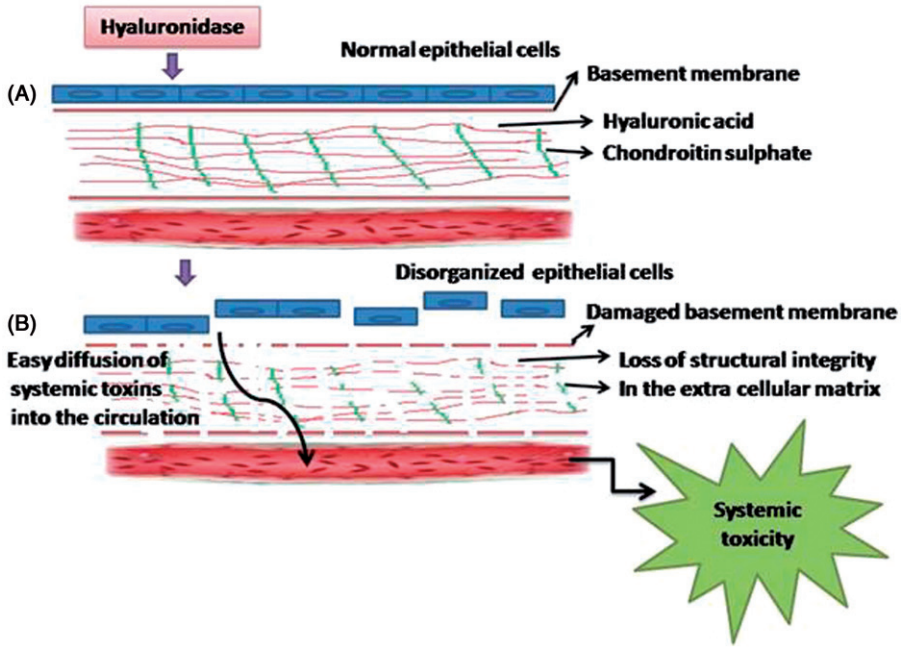


Figure 3. Mechanism of action of hyaluronidase. (A) Intact basement membrane. (B) Damaged basement membrane due to the hydrolysis of hyaluronic acid/chondroitin sulphate by hyaluronidase that cause loss of structural integrity in the extracellular matrix that in turn facilitates the easy diffusion of other systemic toxins into the circulation ultimately leads to the systemic toxicity.

identified hyaluronidase with a molecular weight of ~39 kDa, as a major constituent of venom from the tarantula *Dugesiella hentzi*. Subsequently, hyaluronidase activity was reported from the venoms of spiders *Cupiennius salei* (Kuhn-Nentwig et al., 1994), *Lycosa godeffroy, Lympona cylindrata/murina* (Rash & Hodgson, 2002), *Loxosceles reclusa* (Wright et al., 1973), *L. rufescens* (Young & Pincus, 2001), *L. deserta, L. gaucho, L. laeta, L. reclusa* (Barbaro et al., 2005) and *L. intermedia* (Da Silveira et al., 2007), *Hippasa partita, H. agelenoides* and *H. lycosina* (Nagaraju et al., 2006). Further, the enzyme hyaluronidase (HPHyal) with molecular mass 42.26 kDa was isolated and characterized from *Hippasa partita* venom (Nagaraju et al., 2007a,b). HPHyal belongs to a neutral active group of enzymes as it exhibited high specificity for hyaluronan. Moreover, HPHyal indirectly potentiates the myotoxicity of VRV-PL-VIII (*Vipera Russelli* Venom phospholipolytic enzyme VIII) myotoxin and also the hemorrhagic potency of hemorrhagic complex-I (Nagaraju et al., 2007a,b). Thus, in venoms, hyaluronidase is popularly called a ''spreading factor'' as it facilitates the diffusion of systemic toxins in the circulation of prey (Figure 3). Rapid hydrolysis of the long linear megastructure of hyaluronan into fragments of varied molecular size drastically reduces the viscosity of the envenomed milieu thus favoring rapid diffusion of toxins into circulation which would otherwise diffuse more slowly (Girish & Kemparaju, 2006; Girish et al., 2002). Brazilian spider venom of the *Vitalius dubius* (*Theraphosidae*) displayed high hyaluronidase activity and also showed activity in a band of ~45 kDa in hyaluronan zymography (Rocha-E-Silva et al., 2009a,b). Clement et al. (2012) cloned and expressed hyaluronidase (BvHyal) from the tarantula *Brachypelma vagans* venom. Ferrer et al. (2013) showed that the recombinant hyalurindae from *L. intermedia* venom increased the dermonecrotic effect. Recently, Sutti et al. (2014) characterized hyaluronidase from the venom of *Vitalius dubius* and found that it exhibited similar biochemical and pharmacological properties to previously reported hyaluronidases.

Phospholipases

Phospholipases hydrolyze phospholipids into fatty acids and other lipophilic substances. Depending upon the type of reactions they catalyze, they are classified into phospholipases A, B, C and D. Phospholipase A enzymes are further classified into two categories, Phospholipase A_1 cleaves the SN-1 acyl chain and Phospholipase A_2 cleaves the SN-2 acyl chain. Phospholipase B (lysophospholipase) cleaves both SN-1 and SN-2 acyl chains. Phospholipase C cleaves the acyl chain before the phosphate group and releases diacylglycerol and a phosphate-containing head group. Phospholipase D cleaves after the phosphate and releases phosphatidic acid and an alcohol. Generally, phospholipase C and D are considered phosphodiesterases. Phospholipases from spider venom were found to cause both local and systemic toxicity (Figure 4). Sheumack et al. (1984) for the first time detected phospholipase A activity from the venom of the Australian funnel-web spider *Atrax versutus* (now *Hadronyche versuta*). Subsequently, Usmanov & Nurtova (1994) identified an anticoagulant phospholipase A enzyme in the venom of *Eresus niger* spider venom. For the first time, da Silveira et al. (2006) purified Phospholipase D from *L. laeta* spider venom and it was found to inhibit the aggregation of human platelets. In addition, Phospholipase D was found to act on sphingomyelin and glycerophospholipids that could generate bioactive mediators, namely ceramide-1-phosphate and lysophosphatidic acid, which play a key role in various pathological and biological reactions (Lee & Lynch, 2005). Based on the classification made by Murakami et al. (2006), there are two families of spider-venom phospholipases D. The class-I family has a single disulfide bridge along with an extended hydrophobic loop, whereas the class II

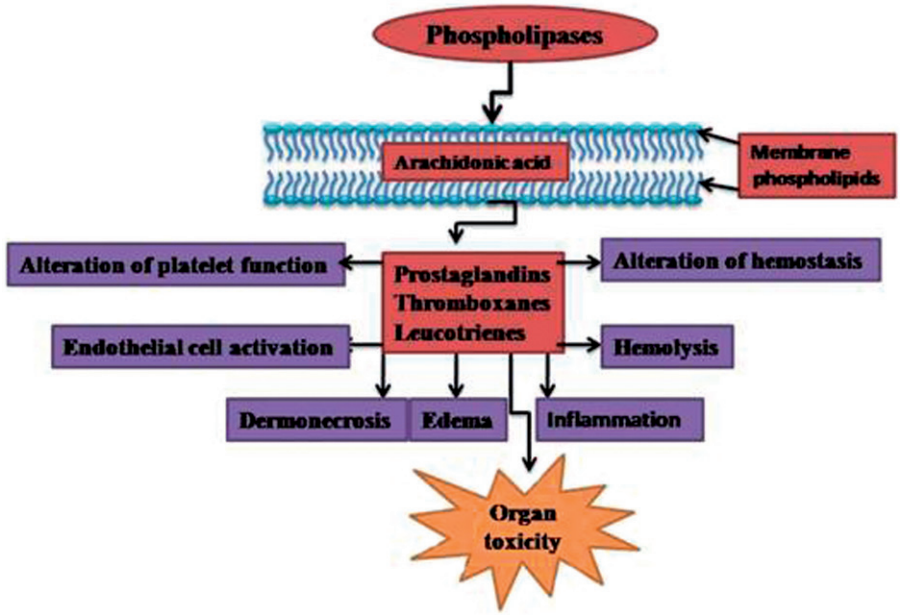


Figure 4. Pharmacological properties of spider-venom phospholipase. Phospholipase hydrolyze arachidonic acid into prostaglandins, thromboxane and leucotrienes as the end products. These end products cause organ toxicity, inflammation, hemolysis, edema, dermonecrosis and alter hemostasis.

family phospholipases D have a the supplementary intrachain disulfide bridge and exhibit limited catalytic activity towards phospholipids (Appel et al., 2008; Chaim et al., 2011; Chaves-Moreira et al., 2009; Gremski et al., 2014; Inoue et al., 2009; Ullah et al., 2011; Vuitika et al., 2013). Catalán et al. (2011) reported on the recombinant phospholipase D isoforms such as rLIPLD1 and rLIPLD2 from the venom of the spider *L. laeta*. However, only recombinant rLIPLD1 hydrolyzed sphingomyelin and showed hemolytic activity, while rLIPLD2 did not. Further, polyclonal antibodies raised against the isolated recombinant proteins (polyclonal anti-rLIPLD1 and rLIPLD2) exhibited immunoprotection against the venom. Thus, these findings suggest potential therapeutic applications of these antibodies in envenomation by the *L. laeta*. Recently, Magalhães et al. (2013) cloned and expressed phospholipase D (LgRec1) from the venom gland of *L. gaucho*. LgRec1 was found to cause local toxic effects such as edema, erythema, ecchymosis and paleness, dermonecrosis and hemolysis. In addition, it hydrolyzed sphingomyelin and promoted platelet aggregation.

Proteases

Proteases are a very important group of enzymes; they specifically hydrolyze the peptide bonds of proteins. These enzymes are present in all organisms. Proteases are involved in several vital physiological functions such as digestion, the blood coagulation cascade; complement system and apoptosis pathways (Kini, 2005). Proteases are broadly classified as exopeptidases and endopeptidases. Those cleaving the peptide bond from the N- or C-terminus end are exopeptidases (aminopeptidases and carboxypeptidases) and those cleaving internal peptide bonds are endopeptidases. The endopeptidases (EC 3.4) are further classified into metallo, serine, cysteine and aspartate family proteases. Kaiser (1956) was the first to report proteolytic activity from the venoms of *L. raptorial* and *C. nigriventer* spiders. Spider venom was reported to contain both metallo and serine proteolytic activities (Feitosa et al., 1998; Veiga et al., 2000). Devaraja et al. (2008) for the first time purified and characterized serine protease from *Hippasa agelenoides* spider venom gland extract. Figure 5 represents the mechanism of action of proteases and their pharmacological properties.

Metalloproteases

Metalloproteases are enzymes that depend completely on metal ions for activity. Most of these enzymes are zinc-dependent; some of them also use cobalt for their catalytic function. Metalloproteases are known to cause several pharmacological effects such as hemorrhage, edema, dermo/myonecrosis and hemostatic disturbances (Nagaraju et al., 2007a,b, 2011). Metalloproteoase activity in spider venoms was first reported by Feitosa et al. (1998) from *L. intermedia*. Subsequently, metalloprotease activity was reported in several spiders such as *L. refescens, L. godeffroyi, Lampona cylindrata/murina, L. intermedia, L. deserta, L. gaucho, L. laeta, L. recluse* and *H. partita* (Atkinson & Wright, 1992; da Silveira et al., 2002; Nagaraju et al., 2007a,b; Pincus et al., 1999; White et al., 1995; Young & Pincus, 2001; Zanetti et al., 2002). So far, two spider-venom metalloproteases have been purified one each from *L. intermedia and H. partita* venom (Nagaraju et al., 2007a,b; Zanetti et al., 2002). da Silveira et al. (2007a,b,c) cloned and expressed a 30 kDa *Loxosceles* astacin-like protease (LALP) and it showed cross-reactivity in the region of a 29-kDa native venom protein. Further, the LALP degraded fibrinogen, fibronectin and gelatin. The members of astacin-like family metalloproteases such as, LALP2, LALP3, LALP4 and LALP5 with gelatinolytic activity were cloned and found to be expressed in venoms of *L. laeta* and *L. gaucho* (Trevisan-Silva et al., 2010). Partitagin, a Zn-dependent hemorrhagic metalloprotease in addition to dermo- and myonecrotic effects exhibited fibrin(ogen)olytic activity and inhibited collagen-induced platelet function (Nagaraju et al., 2011). Recently, Trevisan-Silva et al. (2013) reported on the differential

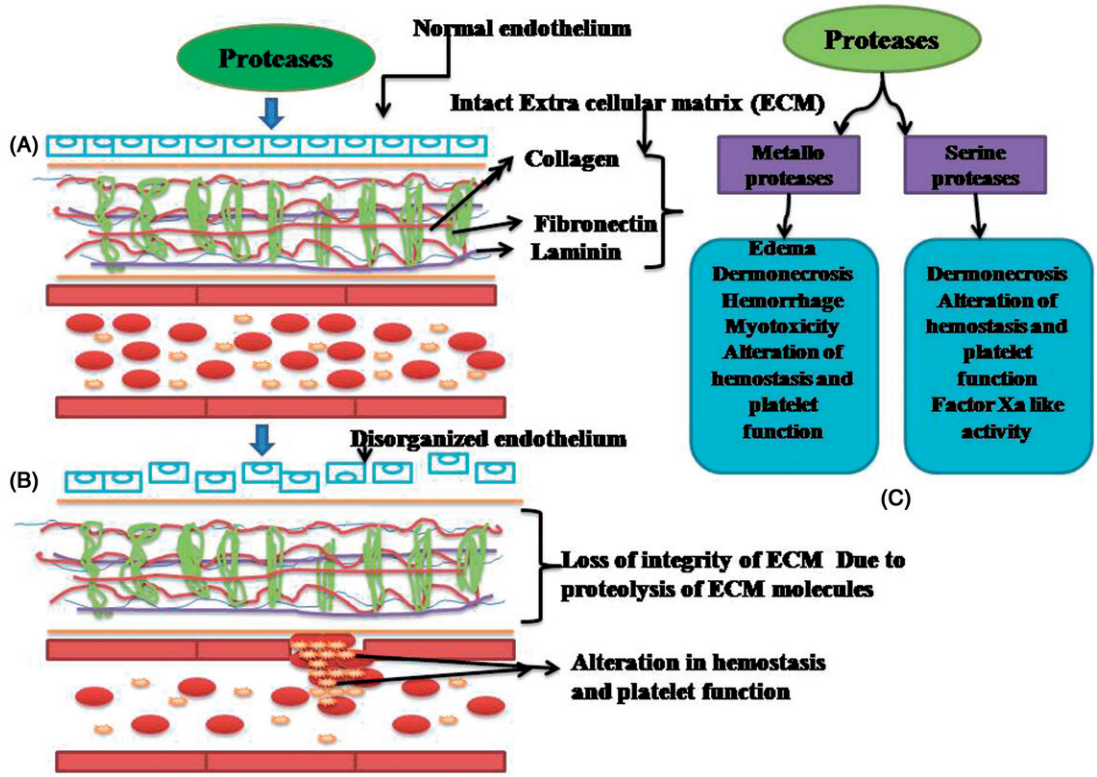


Figure 5. Mechanism of action of proteases. (A) Intact extracellular matrix. (B). Loss of integrity of extracellular matrix due to the proteolysis of collagen, fibrinonectin by proteases that facilitates easy entry of toxins into circulation that further leads to the alterartion in hemostasis and platelet function. (C) Pharmacological properties of proteases.

metalloprotease content of three *Loxosceles* spiders venom capable of hydrolyzing gelatin and fibrinogen.

Serine proteases

Despite a long history of the proteolytic activity of spider venom (Kaiser, 1956), serine proteases are the least explored enzymes of spider venom. Serine proteolytic activity was first identified by Veiga et al. (2000) from *L. intermedia* venom using zymography experiments. However, Devaraja et al. (2008) for the first time reported on the purification, biochemical and pharmacological characterization of a low molecular weight (16.35 kDa) serine protease (Hag-protease) from a venom gland extract from *Hippasa agelenoides*. It dose dependently hydrolyzed proteins such as casein, fibronectin, collagen type-I, human fibrinogen and fibrin but did not degrade gelatin and collagen type-IV. It was non-lethal and devoid of hemorrhagic, myotoxic and edema forming activities. However, Hag-protease interfered in plasma coagulation; the procoagulant property of Hag-protease was due to its factor Xa like activity (Devaraja et al., 2010). Furthermore, at low concentration, the Hag-protease stimulated aggregation of human platelets in platelet-rich plasma; in contrast, it inhibited collagen-induced aggregation of washed human platelets (Devaraja et al., 2010). In addition, medium molecular weight (28.749 kDa) serine proteases the hag-protrase-II was also purified and characterized from *Hippasa agelenoides* spider venom gland extract (Devaraja et al., 2011). It also hydrolyzed casein, fibrinogen, and fibrin but did not degrade gelatin and the extracellular matrix molecules such as, fibronectin and collagen types I and IV. It was non-lethal and devoid of hemorrhagic, myotoxic and edema forming activities. It did not cause tissue destruction. However, it reduced the plasma re-calcification time of citrated human plasma and coagulated the factor X deficient congenital human plasma. It alone showed the weak aggregation response of human platelets in platelet-rich plasma while it did not interfere in collagen-induced aggregation of platelet-rich plasma and washed human platelets (Devaraja et al., 2011).

Collagenases

Collagenases are enzymes that break the peptide bonds in collagen. Studies on the enzymatic activity of venom from several species of spiders have failed to detect the presence of collagenase activity (Kaiser & Raab, 1967; Schenone & Suarez, 1978; Suarez et al., 1971; Wright et al., 1973). However, Atkinson & Wright (1992) reported the presence of collagenase activity in the Australian spiders *Nephilia edulis, Eriophora transmarine* and *Isopeda immanis*. Nagaraju et al. (2007a,b) isolated and characterized a hemorrhagic zinc-dependent metalloprotease ''Partitagin'' that specifically degrade collagen type-IV but not collagen type-I. However, A low molecular weight serine protease (Hag-protease) purified from *H. agelenoides* spider venom, was found to specifically degrade collagen type-I but not collagen type-IV (Devaraja et al., 2008).

Phosphodiesterases

Phosphodiesterases are a group of enzymes that breaks a phosphodiester bond. Russel (1996) for the first time reported

phosphodiesterase activity in venom of *Aphonopelma robustus, A. cratus* and *Latrodectus mactan*. Phosphodiesterases are metallo enzymes containing zinc as a prosthetic group and their activity was activated by magnesium. The pharmacology of phosphodiesterase is unknown. However, they may cause hypertension by generating purins.

Alkaline phosphatases

Alkaline phosphatases are involved in the removal of phosphate groups (dephosphorylation) from nucleotides, proteins and alkaloids. Heitz & Norment (1978) detected alkaline phosphatase activity from the venom of *L. reclusa*. Later, Norment & Foil (1979) reported alkaline phosphatase activity. Rodrigues et al. (2006) for the first time isolated and characterized acid and alkaline phosphatases from *Parawixia bistriata* spider venom. The enzyme showed maximum activity up to 55 °C at the optimum pH range from 5.0 to 8.5 suggesting its acid and alkaline phosphatase activities, respectively. Although pharmacology of spider venom alkaline phosphatase is not established, their distribution in venom clearly suggests the key role in envenomation.

ATPases

These enzymes hydrolyze ATP and releases adenosine and pyrophosphate as the reaction products. Geren et al. (1976) detected ATPase activity from the venom of *L. recluse*. Later, Schenone & Suarez (1978) showed ATPase activity from the venom of *L. laeta*. Although, the ATPase enzymes were detected in the spider venoms, their biological properties are not studied in detail.

Peptide isomerases

Peptide isomerases are least studied enzymes of the spider venom. However, Shikata et al. (1995, 1998) isolated and characterized a novel peptide isomerase, which specifically inverts the chirality of Ser 46 of a 48-amino-acid peptide from *A. aperta* spider. However, the pharmacology of peptide isomerases is unknown.

Non-enzymatic toxins

Translationally controlled tumor protein

The translationally controlled tumor protein (TCTP) or histamine releasing factors were identified in the venoms of *L. intermedia, laeta and Hogna aspersa* spiders with the aid of transcriptomic approaches (Gremski et al., 2010). Interesting point is that the TCPT proteins of *L. intermedia* and *L. laeta* venom gland trancriptome showed 97% sequence homology. Although physiological functions of TCPT are not yet explored, upon envenomation TCPT proteins promote the histamine release in basophils and induce the production of interleukins from basophils and eosinophils (Bheekha-Escura et al., 2000; Sun et al., 2008). Studies by various research groups suggested that histamine released by TCTP proteins binds to their receptors there by induce edema, increase vascular permeability and vasodilation (Chaim et al., 2011; Weisel-Eichler & Libersat, 2004). Thus, TCTP is also having several therapeutic applications in experimental oncology and in the development of anti-cancer drugs.

Serine protease inhibitors

It is a known fact that protease inhibitors have immense therapeutic applications and all known protease inhibitors are proteins (Neurath, 1989; Rimphanitchayakit & Tassanakajon, 2010). The protease inhibitor was reported in the venom of *L. laeta and later in L. intermedia* (Fernandes-Pedrosa et al., 2008; Gremski et al., 2010), in both of these venoms, the sequence analysis study suggested that they are belong to the Serpin superfamily. Serine protease inhibitor reported from *L. laeta* showed anticoagulant and antithrombotic activities by inhibiting the factor Xa (Gremski et al., 2014). Further, serine protease inhibitor was also identified in tarantula's venoms and it strongly inhibited trypsin and it could also be able to block potassium channel (Yuan et al., 2008). Snake venom serine protease inhibitors are extensively studied compared to spider venom serine protease inhibitors. Thus, much of the effort should be put in to explore serine protease inhibitors from the spider venoms that definitely help in the better management of serpinopathies and coagulation disorders.

Pharmacological properties of spider venom

Even though, the spider bite is an accidental event an envenomation causes several pharmacological effects on the victim. The envenomation is generally characterized by two phases: first, it begins within minutes of the bite, and the second, when the toxins subside typically many hours later. Majority of spiders do not cause lethal effect, only a small group of spiders are capable of producing death in humans (Futrell, 1992; Lung & Mallory, 2000; Williams et al., 1995). The pathophysiology appears to involve a complex series of events that depends on the combined and perhaps synergistic action of the venom toxins (Nagaraju et al., 2006). The pathophysiology includes ''Local toxicity'' (edema, hemorrhage, myo/dermonecrosis) and ''Systemic toxicity'' (neurotoxicity, cytotoxicity, cardiotoxicity, myotoxicity, interference in coagulation [pro/anti] and platelet function). The spider bite may also produce considerable local tissue necrosis with scar formation and ulcers that may require surgical repair. The victims may suffer from intravascular hemolysis, disseminated coagulation and acute renal failure leading to death (Elston et al., 2000; Futrell, 1992; Lung & Mallory, 2000; Veiga et al., 2001a,b; Zanetti et al., 2002).

Local toxicity

The early symptoms of the spider bite are the local changes or the local toxicity that takes place within 6–8 min and may have onset up to 30 min. The venom of *Loxosceles* and Australian funnel-web spider takes more than 30 min to cause local reactions. The Australian spiders belong to the genus, *Latrodectus, Steatoda, Sparassidae, Lycosidae, Lamponidae and Mygalomorphae* were found to cause pain, redness, swelling, diaphoresis and itching (Isbister & White 2004). According to the literature, approximately 10 000 spider bites are reported annually in Brazil, 11 species belongs to the

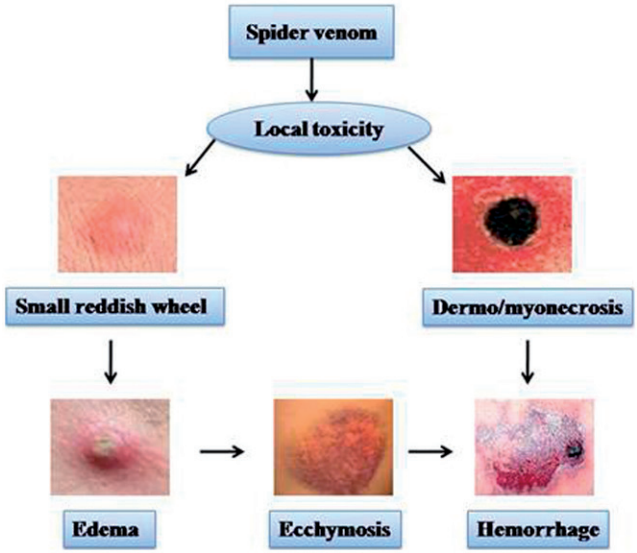


Figure 6. Local toxicity triggered by spider venom.

genus *loxosceles* (mainly *L. laeta, L. gaucho, L. intermedia*) and causes dermonecrosis (Dantas et al., 2014). The North American spiders belongs to the genus *Agelenopsis, Araneus, Cheiracanthium, Hololena, Latrodectus, Eratigena* causes pain, swelling, itching, redness and edema (McKeown et al., 2014). The spiders of the genus *Hippasa (H. partita, H. agelenoides* and *H. lycosina*) belongs to the Western Ghats of Karnataka, India, found to cause severe edema, itching, acute pain and sometimes hemorrhage following tissue necrosis in farm and plantation workers (Nagaraju et al., 2006). In general, a small reddish wheal forms at the bitten region along with swelling that results in edema, echymoses (pinpoint red spots on the skin), hemorrhage and dermo/myonecrosis that are usually visible within minutes of the bite. The local area of the bite may become devascularized with features of necrosis predisposing to onset of gangrenous changes. Secondary infection, including formation of an erythematous area, inside of which is a pale ischemic region that develops a dark necrotic center as the lesion matures. Healing is slow, and these ulcers may persist for months leaving a deep scar represents the local toxicity triggered by spider venom.

Edema

Normally, the amount of interstitial fluid is in the balance of homeostasis. Increased secretion of fluid into the interstitium or impaired removal of this fluid may cause edema. Edema formerly known as dropsy or hydropsy is the increase of interstitial fluid in any tissue or organ. Generation of interstitial fluid depends on the balance of osmotic pressure and hydrostatic pressure, which act in opposite directions across the semipermeable blood capillary walls. Consequently, anything that increases oncotic pressure (exerted by tissue fluid proteins) outside blood vessels (e.g. inflammation), or reduces oncotic pressure in the blood (states of low plasma osmolality, e.g. cirrhosis) will cause edema. Several enzymatic and non-enzymatic toxins from various venoms have been shown to cause edema. PLA_2 mediated arachidonate end products (Vadas & Pruzanski, 1984), oligosaccharides of hyaluronic acid degradation products (de la Motte, 2003; Noble, 2002; Toole, 2004) are found to be highly edematic. Cleavage of cysteine-rich domains of proteins results in the formation of edema forming peptides (Teixeira et al., 2005). The whole venoms of *H. partita* and *H. lycosina* spiders found to cause edema in the footpads of experimental mice (Nagaraju et al., 2006). While the venom gland extract of *Hippasa agelenoides* showed weak edema in the footpads of experimental mice (unpublished data).

Hemorrhage

Hemorrhage is the medical term for bleeding. In common usage, a hemorrhage means severe bleeding, although, technically it means the escape of blood to extravascular space, with the damage to the micro vessel of blood wall. Predominantly, zinc dependent metalloproteases of ''metzincin'' family enzymes are responsible for the hemorrhage induced by the venoms. Metalloproteases generally degrade the extracellular matrix molecules of the endothelium and cause hemorrhage. Most of the spider venom including *Loxosceles* species, *H. partita* and as well as purified ''Partitagin'' from *H. partita* spiders are found to cause hemorrhage (Nagaraju et al., 2007a,b; Veiga et al., 2001a,b). ''Partitagin'' is a non-myotoxic but hemorrhagic metalloprotease purified from *Hippasa partita* spider venom, it damaged the basement membrane of blood vessels in the skin and annihilated the integrity of muscle by degrading the extracellular matrix of the muscle tissue but not the myocytes (Nagaraju et al., 2007b).

Dermo-/myo-necrosis

The loss of integrity of extracellular matrix molecules due to the degradation of extracellular matrix degrading venom enzymes is an important process in the development of dermo-/myo-necrosis. In addition to hemorrhagic metalloproteases, hyaluronidases and myotoxic phospholipases, sphingomyelinase D are also implicated in inducing dermo-/myo-necrosis (de Souza et al., 1998; Futrell, 1992). The whole venom of *L. intermedia* and the purified enzymes such as sphingomyelinase D and phospholipase D from *Loxosceles reclusa* were found to cause dermonecrosis (da Silveira et al., 2006; Veiga et al., 2001a,b). This enzyme has been detected in *Loxosceles* venoms like *L. gaucho, L. intermedia, L. rufescens, L. laeta* by a number of research groups (Tambourgi et al., 1998). The enzyme has been purified from the *L. reclusa* and *L. intermedia* venoms. It has been shown to reproduce the major manifestations of *Loxoscelism* (Kurpiewski et al., 1981; Tambourgi et al., 1998). Biochemical analysis of native purified sphingomyelinase-D toxin from *L. reclusa* showed four immunologically cross-reactive isoforms (of 32 kDa having pI value of 8.7, 8.4, 8.2 and 7.8 by isoelectrofocussing) that hydrolyzed sphingomyelin; these isoforms were able to develop dermonecrosis in rabbits. Two sphingomyelinases LiRecDT2 (1062 bp cDNA) and LiRecDT3 (1007 bp cDNA) were cloned, expressed and purified from the venom gland of *Loxosceles intermedia* and they have a molecular weight of 33.8 and 34.0 kDa, respectively. These showed differential action on rabbits: LiRecDT2

caused a macroscopic lesion with gravitational spreading upon intradermal injection, while LiRecDT3 evoked transient swelling and erythema upon injection site. Differential functionality for recombinant toxins was demonstrated by the high level of sphingomyelinase activity for LiRecDT2 and low activity for LiRecDT3 as well as greater *in vitro* platelet aggregation and blood vessel permeability induced by LiRecDT2 and residual activity for LiRecDT3 (Tambourgi et al., 1998). In addition, several non-enzymatic peptides involved in tissue necrosis were reported. A 6.7 kDa necrotoxic peptide appears to be the predominant toxic component of the venom from the female Arkansas tarantula *Dugesiella hentzi* (Lee et al., 1974). The toxin was found to bring about histological changes at the site of injection and in the heart, where it produced the typical lesions. *Phoneutria nigriventer* venom in addition to the neurotoxic effect, also cause smooth muscle contraction (Antunes et al., 1993) and increase the vascular permeability of rabbit skin and rat skin by activating local tissue kallikrein–kininogen–kinin system. A series of peptides with activity accounting for these actions on smooth muscle have been isolated (PNV1, PNV2, PNV3 and PNV4; Bento et al., 1993, 1995; Marangoni et al., 1993; Rego et al., 1996). Sosnina et al (1990) isolated two peptides from the venom of the European widow spider *Lactrodectus tredecimguttatus* that inhibited the activity of Angiotensin converting enzyme. A deca-peptide (BPP-S) acting on the smooth muscle and inhibiting angiotensin-converting enzyme have been isolated from the venom of *Scaptocosa raptorial* (Ferreira et al., 1996). The two kinin-like peptides (peptide-S and peptide-R) which have been isolated were equipotential with bradykinin in contracting guinea pig isolated ileum. The lycotoxins (I and II) are two peptides isolated from the venom of the North American wolf spider *Lycosa carolinensis* with broad range of actions (Yan & Adams, 1998). In insect body wall muscles, lycotoxin I caused complete loss of cell membrane potential and blockade of neuromuscular transmission. Both lycotoxin I and II caused efflux of calcium ions from, and prevented sequestration of calcium ion by rat synaptosomes. In addition, both peptides possess potent antimicrobial activity against both prokaryotic and eukaryotic cells in plate growth inhibition assays. Recently, five low molecular weight antibacterial peptides (3–4 kDa) have been isolated from the venom of *Cupiennius salei* (Haeberli et al., 2000). The venom of Chinese bird spider *Selecosmia huwena* was found to contain a 32 amino acid peptide, which is a lectin-like peptide SHLP-I that agglutinated both mouse and human erythrocytes. ''Partitagin'' a hemorrhagic and non-myotoxic metalloprotease caused severe dermo and myonecrosis without damaging myocytes (Nagaraju et al., 2007a,b). The Hag-protease a low molecular weight serine protease purified from *H. Agelenoides* spider venom gland extract was found to be non-hemorrhagic and non-myotoxic but induces dermo- and myo-necrosis in experimental mice (Devaraja et al., 2008).

Systemic toxicity

The distribution of systemic toxins into their particular targets by the circulating blood leads to systemic toxicity. The systemic toxicity directly depends upon the concentration, efficiency and the rate of diffusion of systemic toxins from the bite site into the blood for distribution (Girish & Kemparaju, 2007). The systemic toxicity includes neurotoxicity, cytotoxicity, myotoxicity, cardiotoxicity, effect on hemostasis, hemolysis and renal failure. Renal failure leads to hemoglobinuria and proteinuria (Devaraja et al., 2008, 2009, 2011; Futrell, 1992; Lung & Mallory, 2000; Nagaraja et al., 2006; Williams et al., 1995). Figure 7 represents the schematic diagram of mechanism action of systemic toxins.

Neurotoxicity

Indeed, spider venoms are rich sources of diverse category of neurotoxin (Davletov et al., 2012; Jackson & Parks, 1989). The primary purpose of the spider venom is to paralyze the prey; hence, the spider venom is a rich source of variety of toxins that affect the nervous system. Predominantly, spider venom neurotoxins characterized to date are found to be proteins/peptides or acyl polyamines. These are found to exert a variety of actions throughout the nervous system. Therefore, majority of spider venom neurotoxins appear to target the neuronal receptors, neuronal ion channels or pre-synaptic membrane proteins that are involved in neurotransmitter release (Davletov et al., 2012). Neurotoxins have been extensively studied from the spider such as, Australian funnel-web spiders, *Atrax robustus, Hadronyche versuta* and the spiecies of *Loxosceles* (Browne et al., 1997; Del Brutto, 2013; Hodgson, 1997; Miller et al., 2000). Versutoxin a neurotoxic peptide (d-ACTX-Hv1a) isolated from *Hadrothemis versuta* and its homologue robustoxin (d-ACTX-Ar1) from *Atrax robustus* were found to cause neurotoxic symptoms in primates (Brown et al., 1988; Mylecharane et al., 1989; Sheumack et al., 1984). Omega-atracotoxins specifically blocked mammalian voltage gated channel, but did not block insects (Wang et al., 1999). *Hippasa partita, H. lycosina, H. agelenoides* spider venom showed neurotoxicity in the experimental animals (Nagaraju et al., 2006). Neurotoxins isolated to date from spider venoms are classified based on their mode of action as: glutamatergic transmission, calcium ion, sodium ion, potassium and chloride ion-channels affecting toxins; toxins that stimulate neurotransmitter release and toxins blocking postsynaptic cholinergic receptors (Cesar et al., 2005; Escoubas et al., 2000; Rash et al., 2002; Reyes-Lugo et al., 2009). Hence, neurotoxins of spiders can be used as potential pharmacological and therapeutic tools (Davletov et al., 2012; King et al., 2013; Klint et al., 2012; Vassilevski et al., 2010; Wong et al., 2013).

Cytotoxicity

Spider venoms were found to cause cytotoxicity on different cell lines. *Loxosceles recluse* venom was cytotoxic to human neutrophils; at higher concentrations it inhibited complement-devised chemotaxis of neutrophils (Majeski et al., 1977). Of the 26 species of spider venoms studied for cytotoxicity, *Araneidae, Lycosidae* and *Oxypidae* family venoms were found to be cytotoxic to insect cell line Sf9 (Cohen & Quistad, 1998). Venom from the jumping spider (Salticidae species) was found to be more cytotoxic for insect Sf9 and mouse N1E-115 cell lines with IC_{50} values less than 1 µg venom protein in both the cases. The brown spider venom (*Loxosceles* sp.) was cytotoxic to rabbit blood vessel

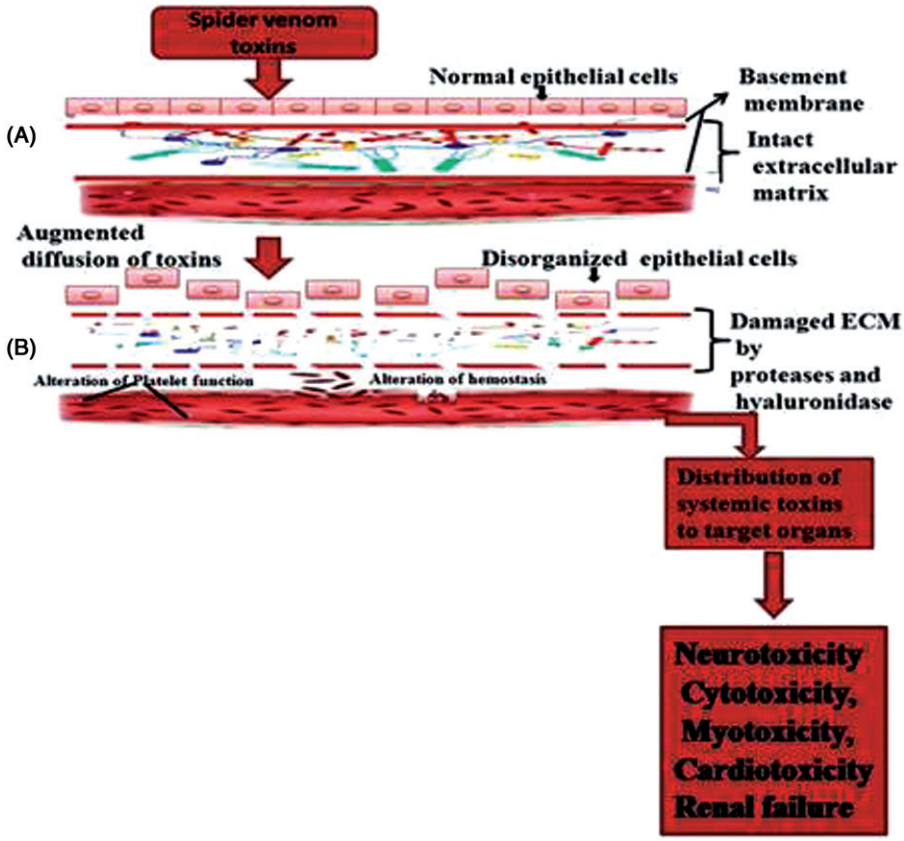


Figure 7. Mechanism action of systemic toxins. (A) Intact basement membrane. (B) Damaged extracellular matrix (ECM) by proteases and hyaluronidases that augment the easy diffusion of systemic toxins into the circulation which in turn distributes systemic toxins to the target organ leads to organ toxicity.

endothelial cells (Veiga et al., 2001a,b), human umbilical vein endothelial cells (HUVECs; Patel et al., 1994) and endothelial cells from skin blood vessel walls of rabbits (Zanetti et al., 2002). Luciano et al. (2004) showed that the envenomation by *Loxosceles* spiders leads to nephrotoxicity in which the toxins bind to the glomerular membrane, tubule cells and basement membranes of kidney cells, leading to the glomerular epithelial cell injury, endothelial cell cytotoxicity, hyalinosis and proteinuria as well as tubule cell damage. This was partly justified due to the action of protease present in the venom. Although these components were not isolated and characterized, this introduced novel activity of spider venom, which may be in part due to enzymes or non-enzymatic toxins present in the venom and reveals the probable consequence of envenomation. *Hippasa partita, H. lycosina, H. agelenoides* spider venoms were cytotoxic to mouse Ehrlich Ascites tumor cells (Nagaraju et al., 2006). CpTx 1 from yellow sac spider exhibited insecticidal, cytotoxic, and membrane-damaging activities (Vassilevski et al., 2010). Cytolytic paptide cupiennin 1a, purified from the *Cupiennius salei* exhibits bacterial membranolytic activity and cytolytic activity towards human blood and cancer cells (Kuhn-Nentwig et al., 2011) Chaves-Moreira et al. (2011) reported that brown spider venom phospholipase-D toxin causes hymolysis.

Myotoxicity

Myotoxicity refers to the damage of skeletal myofibrils due to the action of matrix metalloproteases, Phospholipase A_2 and the neurotoxic peptides of the spider venom (Nagaraju et al., 2006). Toxin PhTx 1 from *Phoneutria nigriventer* was lethal to mice causing neurotoxic symptoms upon i.v. injection (Rezende et al., 1991), it induced morphological changes in nerves and 20–30% of muscle fibers (damaged mitochondria and sarcoplasmic reticulum) suggesting both neurotoxic and myotoxic actions, although the mechanism remains unclear (Mattiello-Sverzut et al., 1998). A myotoxic peptide Covalitoxin-I was isolated from the venom of the Singapore tarantula *Corecnemius validus* that caused necrosis of mouse skeletal muscle (Balaji et al., 1999). The venom of spiders such as *Hippasa partita, H. lycosina, H. agelenoides* are capable of elevating cytoplasmic markers such as lactate dehydrogenase and creatinine kinase in experimental animals suggesting their myotoxic properties (Nagaraju et al., 2006).

Cardiotoxicity

The venom from the Brazilian spider *Lasiodora* sp. (*Mygalomorphae*, and *Theraphosidae*) caused a dose-dependent bradycardia in the isolated heart, with transient cardiac arrest and rhythm disturbances. The effects were found to be reversible upon wash out of the venom. The mechanism suggests that *Lasiodora* venom evokes the vesicular release of acetylcholine from parasympathetic nerve terminals in heart by activating TTX-resistant (tetradotoxin resistant) Na^+ channels. *Lasiodora* venom found to contain several cardiotoxic peptides (Kalapothakis et al., 2003). The whole venom, recombinant toxin (rLiD1) and sphingomyelinase from *Loxosceles intermedia* spider showed cardiotoxic effect and caused impairment of heart function (Dias-Lopes et al., 2010). *Acanthoscurria paulensis (Theraphosidae)* spider

venom was found to arrest the diastolic function of the frog heart (Mourao et al., 2013).

Interference in blood coagulation

Hemostasis is a significant physiological event that arrests bleeding. It comprises a set of sensitive phase of reactions due to vascular injury that activates several clotting factors and platelets to form a solid thrombus which avert the blood loss. The process of blood clot formation either through intrinsic or extrinsic pathways and then the successive dissolution of the clot followed by repairing of the injured tissue are collectively called hemostasis (Kini, 2005). Spider venom components were found to interfere with hemostasis, some promoting coagulation and are called pro-coagulants and others inhibiting coagulation and are called anti-coagulants (Devaraja et al., 2010). Venom from the brown spider (*Loxosceles* genus) showed anticoagulant property upon incubation with citrated human plasma (Zanetti et al., 2002). Partitagin, a hemorrhagic metalloprotease isolated from the venom of funnel web spider *H. partita* caused anticoagulation by citrated human plasma upon exhaustive incubation for a period of 1 h (Shivaiah et al., 2011). A low molecular weight serine protease, the Hag-protease purified from *H. agelenoides* spider venom gland extract caused pro-coagulation of citrated human plasma (Devaraja et al., 2010). In addition to interfering with plasma re-calcification time of citrated human plasma, spider venoms and their purified components were found to exhibit fibrin(ogen)olytic activities. The venoms from *L. interemedia, L. deserta, L. gaucho, L. laeta* and *L. recluse* spiders were found to degrade human fibrinogen in zymography experiments. While, *L. intermedia, H. partita* and *H. agelenoides* spider venoms were shown to degrade Aα or Bβ or both the chains but not the γ-chain of fibrinogen (Nagaraju et al., 2006; Rafael et al., 2002). An astacin-like metalloprotease from *L. intermedia* venom that specifically degrades Aa- and Bb-chains but not the γ-chain was cloned and expressed (da Silveira et al., 2007a,b,c; Veiga et al., 2000; Zanetti et al., 2002). In addition to anticoagulant effect, the Partitagin isolated from the venom of funnel web spider *H. partita* was preferentially degraded Bβ and γ-chains of fibrinogen and γ–γ dimer of fibrin clot (Nagaraju et al., 2011).

Platelet aggregation

Platelet aggregation is an important event of primary hemostasis. Platelet aggregation also contributes significantly to thrombus formation. At resting stage, platelets are in discoid shape in the circulating blood. Hence, at this shape, platelets do not voluntarily adhere to any surface and circulate freely to survey the integrity of inner lining of blood vessels. Perhaps, if there is any damage to the vascular endothelium, platelets become sticky and aggregate at the sites of newly exposed proteins such as collagen and von Willebrand factor (vWF) and form hemostatic plug, that seals the breakage in the blood vessel. Above all, platelets also become sticky upon stimulation by various agonists such as ADP, arachidonate, serotonin and epinephrine; enzymes such as thrombin and trypsin; particulate materials such as collagen and antigen–antibody complexes; lipids, such as platelet activating factor (PAF-acether) and ionophores such as A23187 (Zucker, 1989). Stimulation by these diverse agonists initiates a series of cellular responses such as adhesion, change in platelets shape from disc to sphere and release of various substances (Kini & Evans, 1990). Spider venom toxins alter the platelet function by activating or inhibiting agonist-induced platelet aggregation process. *L. intermedia* venom induced the platelet aggregation and resulted in thrombocytopenia (reduced platelet count) and thrombosis (formation of blood clot) of the dermal blood vessels. The sphingomyelinase-D and phospholipase-D enzymes respectively from *L. intermedia* and *L. laeta* spider venoms were found to activate the platelet aggregation of platelet-rich plasma (Cunha et al., 2003; da Silveira et al., 2006). In addition, two recombinant dermonecrotic toxins from the same venom LiRecDT1 and LiRecDT2 induced platelet aggregation (da Silveira et al., 2006). The Partitagin, an hemorrhagic metalloprotease from *H. partita* venom caused inhibition of collagen-induced aggregation of human platelets (Shivaiah et al., 2011). *Hippasa agelenoides* spider venom gland extract and low molecular weight serine protease the Hag-protease from *H. agelenoides* spider venom gland extract found to aggregate human platelets in platelet-rich plasma and washed platelets (Devaraja et al., 2010). Hag-protease alone induced platelet aggregation and inhibits agonist collagen-induced platelet aggregation of washed human platelets with the IC50 value of 0.29 nmol/ml PRP (Devaraja et al., 2010). In contrast, the Hag-protease-II alone at low concentration caused aggregation of platelet-rich plasma (PRP). However, at the increased concentrations aggregation property of the protease was strongly hindered from the concurrent fibrin clot formation. While, it did not exhibit any influence on the agonist collagen-induced aggregation of platelet-rich plasma and washed human platelets (Devaraja et al., 2011). Partitagin a zinc-dependent metalloprotease inhibited collagen-induced platelet aggregation (Shivaiah et al., 2011).

Therapeutic applications of spider toxins

Currently, toxins from various venomous organisms such as snakes, leech, cone snails and lizards, honey are gaining much importance due to their immense biological/biotechnological applications as they are being used as therapeutic and diagnostic tools (King et al., 2011). For instance, Captropril from Pit viper, Bivalirudin from leech and Exenatide from lizard are the approved drugs used to treat hypertension, anticoagulation during surgery and type 2 diabetes, respectively (King et al., 2011). Although, to date, none of the spider venom toxins are in the market as an approved drug. However, with the development of novel molecular biology techniques, researchers could able to explored biotechnological applications of spider venom components remarkably (Baron et al., 2013; Davletov et al., 2012; King & Hardy, 2013; Klint et al., 2012; Min et al., 2013; Nimmrich et al., 2012; Nunes et al., 2013; Vassilevski et al., 2011; Windley et al., 2012). The omega atracotoxin from Australian funnel web spider venom could be used as a pesticide (Bailey & Wilce, 2001). The omega-ACTH-Hvt1 toxin from *Hadronyche versuta* venom was found to defend the tobacco plant against insects. In addition, spider venom components could also be used as

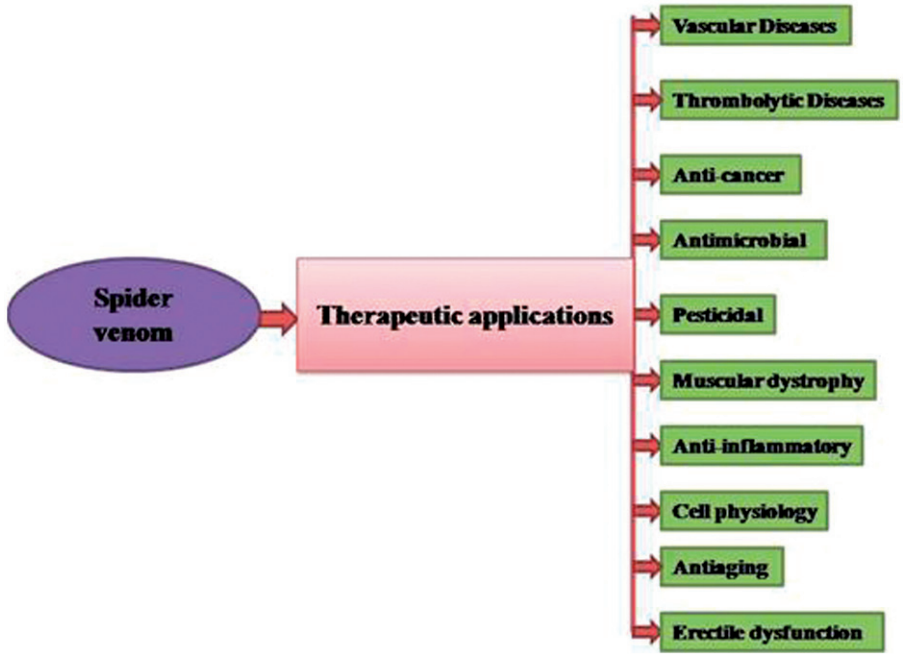


Figure 8. Therapeutic applications of spider venom.

model systems for designing novel therapeutic agents. Tx2–6 toxin found to improve the nitric oxide level in the penile tissue of experimental animals (Andrade et al., 2008; Villanova et al., 2009). Several antibacterial peptides were reported from various spider venoms, the antibacterial peptide from the venom of the *Cupiennius salei* spider acts channel-forming toxins within the bacteria wall (Bailey & Wilce, 2001; Haeberli et al., 2000). ARACHnase is normal plasma which contains *L. recluse* crude venom that mimics the presence of a lupus anticoagulant (Gremski et al., 2014). The phospholipase D is found to activate voltage-gated channels and hence, it could be useful in studying channelopatheis (Ramu et al., 2008). Antiserum based products such as, anti-arachnid serum (obtained using the venom of *L. gaucho*) and anti-*Loxosceles* serum (obtained using the *L. intermedia, L. gaucho* and *L. laeta crude* venoms) could be used in the treatment of spider bite (Pauli et al., 2009). A recombinant phospholipases D from brown spider could be used as putative models for the application in the different areas of cell biology, immunology, pharmacology and biochemistry (Chaim et al., 2011). Inhibitory cystine knot (ICK) peptides from brown spider venom exhibits potential insecticidal effect, hence they could be used as an insecticidal agents (Matsubara et al., 2013). In addition, ICK peptides could also be used to investigate ion channel structures and functions (Klint et al., 2012). Spider venom hyaluronidases could be used in identifying hyaluronidase inhibitors. This could further help in the development of numerous therapeutic agents such as contraceptives, anti-tumor, anti-microbial, anti-venom, anti-wrinkle, anti-aging and inflammation suppressors (Barla et al., 2009; Botzki et al., 2004; da Silveira et al., 2007a,b,c). Thus, spider venom hyaluronidases are also having several potential applications (Calvete, 2009; Escoubas et al., 2000; Gremski et al., 2010; Senff-Ribeiro et al., 2008). TCTP can be used to study tumor cell behavior, cell cycle regulation, cell physiology, organelle transport and designing anti-cancer agent as well. The metallo and serine proteases can be used in the treatment of vascular diseases (acute myocardial infarction, acute ischemic stroke, thrombosed aortic aneurysms, pulmonary embolism, etc.) and as thrombolytic agents (da Silveira et al., 2007a,b,c; Trevisan-Silva et al., 2010). Figure 8 represents the therapeutic applications of spider venom.

Conclusion

Spider venom epitomizes the wide cocktail of genuine pharmacologically active enzymatic and non-enzymatic toxins. The purposes of these toxins are for prey acquisition and defense.

Spider bite is an accidental event that leads to medically significant complications. The most often seen cases of spider bites occur in humans and domesticated animals. Although 98–99% of the bites are harmless, more rarely the symptoms can include necrotic wounds and systemic toxicity. Though several enzymatic toxins were identified in the spider venom, so far only the pharmacology of spingomyelinase, phospholipase, proteases, hyaluronidases have been established. However, non-enzymatic peptides and their therapeutic and biotechnological applications have been extensively studied. Hence, the spider venom toxin research became one of the fascinating fields. However, there are several drawbacks associated with spider venom research. As spider is a tiny organism and available in a specific season, getting venom in sufficient quantity is a tedious job. However, with the currently available developed novel molecular biology techniques, therapeutic applications of non-enzymatic toxins have been immensely reported. While, there is still a lack of studies on the mechanism of action and structure and function relationship of enzymatic toxins. Therefore, it is high time for the biochemist, enzymologist and structural biologist to explore the structure–function relationship of spider toxins for the better understanding of the mechanism of action and their applications.

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