Functions and Therapeutic Applications of Venom Proteins

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Venomous animals are a serious threat to the life of victims both animal and human and 1.45 million people around the world are affected by snake bite. As per the report of Kasturiratne et al. 2008, annually 20,000-94,000 deaths are recorded globally and the main reason is untreated (especially in poor countries) due to expensive of antivenoms and the limitation and complication of the drugs. Targeting the toxins which cause the serious or immediate death can be neutralized by new effective drugs thus can save the victims and large scale production can lead to the easy available to all people. On the other hand, understanding the biochemical property of venom will help us to treat the envenomation, as well as some specific functional toxin molecule with protein engineering, can be developed as a lifesaving drug. This article gives a glimpse of the venom proteins and their functional properties and the list of drugs approved from animal toxins. The overall study focused on the functional specificity of the venom proteins, molecular evolution with their biological importance which can be targeted for treatment and developing drugs from the proteins.

Introduction

Human civilization and venom animals have close contacts and living over the ages and decades. Snakes are very much familiar with our cultural, traditional and spiritual icons like worshiping the snakes (snake worshiping as God/ Gods with snake), wound double snakes and many-headed cobra protecting Buddha and so on. The modern world also has the manifestation of the cultural and social interest with venomous animals like bees, scorpions, spiders, snakes and wasps.

There is a lot of superstition about the snake and its behaviour in India and around the world which the scientific community has to address it to all for avoid the misconceptions and superfluous dreads and adversely they are beneficial to the researchers and the people as the therapeutic drugs after the series of the molecular evaluation process. The venom is toxins in the form of fluid made by the animal is a major component of proteins and less composition of carbohydrate, organic molecules and RNA. Most of the venom is injected to the victim/prey using teeth/fangs but snake venom has vital toxic components and has a more powerful chemical weapon to survive and protect themselves. The majority of venomous animals use their venom for the purpose of hunting the prey with different types of mechanism thus may be the reason for the evolution of different venom delivery and compositions.

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The reports have been suggested that the venom evolved via the recruit and gene duplication events with normal physiological function and their expression elsewhere within the body (Fry, 2005). The event may occur by; (1) transcription-based sequence of oral gland transcript/protein derived from a variety of squamate reptiles, (where venom precursor genes acts as normal housekeeping genes), and (2) the raw material for the evolution of venom toxin is derived from widely distributed genes with normal genes with regular physiological functions. The recent study has suggested that the snake venom has been evolved from pre-existing salivary proteins to the venom gland but not evolved from the tissue or body proteins. At present, it appears like that the venom protein homologs are broadly expressed throughout many tissues of venomous and non-venomous snakes, but the actual genes corresponding to the venom toxins are overexpressed in specifically in the venom gland [1, 2]. Additionally, the molecular phylogenetic analysis of Hyaluronidase reveals that there is a sequence of evolution carried out from the earlier eukaryotic to the non-venomous and venomous snakes simultaneously and the complexity and the functional diversity of the Hyaluronidase within snake species have to be characterized (our unpublished data).

Sting by a bee or a wasp give more pain but cobra biting gets you paralyzed and leads to death also. Different animal's venom has diverse biological/physiological effects due to the composition and type of molecule presence in the venom secretion. For example, the cone snail uses proboscis as sting element and siphon acts as the sensory element to attack and catch a fish through its chemosensory/drug-delivery mechanism. The harpoon-like tooth (hypodermic needle or specialized hook) is a major tool for the fish hunting and then it engulfs and regurgitates the prey. The harpoon has a wide diversity and each time it used a different shape of the needle for catching the fish is the gift of nature.

The animal type and behaviour, geographical region, shape and food habit of the animal play a major role in the type of prey attack and delivery system of the venom. The conus snail produces the conotoxin, a toxic peptide for the attack of the neuromuscular system of the prey, a fish. There is more forms of conotoxin, ω-conotoxin (inhibits voltage-gated calcium ion and acetylcholine), α-conotoxin (impairs the acetylcholine and muscle sodium channel receptor), μ-conotoxin (prevents the muscle action potential) [3].

Snake venom (SV) is a mixture of proteins and peptides that perform a myriad of biological functions [4]. The majority of venomous snakes are found in the Colubridae, Elapidae, and Viperidae families. Viperidae venoms are rich in the enzymes like phospholipase A_2 (PLA_2), Serine proteases (SP), Metalloproteinases (SVMP), L-amino acid oxidases (LAAO), and phosphodiesterases (PDE). In contrast, the non-enzymatic proteins have an essential physiological role like disintegrins, cysteine-rich secretory proteins (CRISPs), Hyaluronidase and C-type lectins [5]. Elapidae venoms are significantly found with a lower concentration of SVMP and SP, lack of disintegrin, and abundant of PLA_2, and fast-acting three-finger neurotoxins [6, 7]. The composition and specific function of the venom proteins determine the physiological effects and the toxicity. The basics of venom protein and their biological function will provide a better understanding of venomous snakes and their toxins.

Venom Protein and Its Functions

There are nearly 24 different protein families are present in reptile venom, with large diverse pharmacological activities. It is observed that some venom protein family have a wide pharmacological function like PLA_2 which exhibits neurotoxic, myotoxic, coagulation and anticoagulant effects. This clearly demonstrates that several animal venom component shows profound species-specific activity, thus leads to rapid immobilization and death of prey.

Toxic Venom Protein
Metalloprotease

SVMPs (Snake venom metalloproteases) are classified based on the various functional domain's presence within the structure [8], where they sharing MP domain that constitutes of the canonical zinc-binding motif (H-E-X-X-H-X-X-G-X-X-H) at the catalytic site, followed by a highly conserved Met turn. In common, SVMP contributes for the proteolytic degradation of membrane and extracellular components (primarily type IV collagen and perhaps perlecan; [9], and they have predominant local and systemic effects very often in victim after the envenomation.

Further, Viperidae family has large diversity of SVMPs by expressing the class of P-I (M-domain; Metalloprotease), P-II (MD-domain; Metalloprotease and disintegrin), P-III (MDC-domains; Metalloprotease, disintegrin and cysteine-rich domain) and P-IV, contain an additional snake C-type lectin-like domain along with P-III [10]. SVMPs play a crucial functional role in prey immobilization, and tissue lysis/proteolysis and they are assisting in the digestion of large and heavy body prey that consumed by Viperidae and some Colubridae snakes in tropical regions [11]. In addition, SVMPs are responsible for hemorrhagic activity and it attributes disparate other activities also. It was reported that they exhibit prothrombin activation (Kini, 2005; Yamada et al., 1996), fibrinolytic (Retzios and Markland, 1988; Willis and Tu, 1988), factor X activation in blood coagulation pathway (Siigur et al., 2004; Tans and Rosing, 2001), apoptotic process (Brenes et al., 2010; Han et al., 2007; Trummal et al., 2005), pro-inflammatory (Gutierrez et al., 1995; Moura-Da-Silva et al., 2007; Rucavado et al., 1995) and inactivation of blood serine proteinase inhibitors (Kress, 1986; Kress and Catanese, 1980; Kress and Hufnagel, 1984).

Phospholipase A_2

Phospholipase A_2 (PLA2; EC 3.1.1.4) cleaves the phospholipids into fatty acids and lysophospholipids by hydrolyzing the ester bond (2-acyl) of 1, 2-diacyl-3-sn-phosphoglycerides [12]. Secretory PLA2s (sPLA2) are low molecular weight (~14 kDa) enzymes containing 6 to 8 disulfide bridges. $sPLA_2$ are mostly found abundance in snake and bee secretory venom mixtures. The enzyme has conserved structural and functional important features with the mammalians $sPLA_2$, like a common catalytic mechanism, calcium cofactor requirement for the activity, and much conserved structural fold.

Phospholipase A_2 (PLA_2) constitutes a major component of snake venom and often contributes to the substantial immobilization of prey and capture by its fast acting. PLA_2 activity and characterization have been studied from many snakes species belong to Viperidae, Elapidae, and Colubridae family [13, 14]. Phospholipases A_2 are among the highly toxic and distinct pharmacologically active venom component, and significant research was performed and still existing more mysteries to be addressed about the enzyme. Snake venom PLA_2 is type of secreted type enzyme and the diversity endured of pre or postsynaptic neurotoxin, cardiotoxin, myotoxin, platelet aggregation inhibitor, pro-coagulation activator, anticoagulation factor and accomplishes hemolysis, edema, convulsion, and hypotension [15-18]. In the recent study, we have reported that the natural compound Piperine was as a prominent inhibitor for the pharmacological functions of *Echis Carinatus* PLA_2 [19].

In some Elapidae species (*Micrurus nigrocinctus*), 48% of its venom proteome is comprised of the PLA$_2$ enzyme (Fernandez et al. 2011) and among the Viperidae, tiger rattlesnake (*Crotalus tigris*) contains approximately of 66% of a homolog of Crotoxin, a presynaptic b-neurotoxic PLA$_2$ (Calvete et al. 2012). Mojave toxin from C. scutulatus is a primary toxic PLA$_2$ in the venom which composed of 45% venom proteins found in southeastern Arizona, but it not observed in nearby north-central Arizona regions (Massey et al. 2012).

Crotoxin is a presynaptic neurotoxic PLA$_2$ was isolated from the venom of *C. d. terrificus* (Horst et al. 1972), and homologs have been isolated from many rattlesnakes (Aird and Kaiser 1985; Aird et al. 1985) and other pit vipers (Lomonte et al. 2015). The presence of these toxins in rattlesnake venoms led to the recognition of type I/type II venoms in vipers (Mackessy 2008, 2010), and those species with venoms containing significant amounts of the toxin are the most toxic (type II venoms). These toxins are non-covalent heterodimers composed of an acidic, non-toxic, non-enzymatic 9.4 kDa subunit, and a basic, moderate toxicity 14.2 kDa PLA$_2$ subunit; the complex is quite potent toward mice model with LD$_{50}$ nearly of 0.03 mg/g (Aird and Kaiser 1985).

Serine Proteases (SP)

Procoagulant SP

All the procoagulants from the snake venoms characterized are proteases and Serine Proteases (SPs) are playing vital role in the coagulation process; SP activates a zymogen of specific coagulation factor who is involved in the coagulation cascade and it quicken the blood clot formation leads to cardiac arrest and other severe effects. For example, RVV-V from *Daboia russelli* and Thrombocytin from *Bothrops atrox* activate the factor V, a cofactor in the prothrombinase complex. Venom metalloproteases also directly involved in activation coagulation protein cofactors like factor V and does the similar function of SP with different mechanism [20].

Snake venom proteins that specifically activates the factor VII is not yet been reported clearly. But, a study shown that Oscutarin (a prothrombin-C activator) was activated the factor VII from *Oxyuranus scutellatus* [21] and the study confirmed that the activation took place in the similar site and it was highly supported by Ca^{2+} ion and phospholipid while interacting with the factor Va-like subunit. Venoms from Viperidae, Crotalidae and Elapidae contain a variety of proteases and they are capable of activating the factor X [22, 23]. But, a few serine proteases activates the factor X which was isolated from the venom of *Ophiophagus hannah*, *Bungarus fasciatus*, *Cerastes cerastes* and *Cerastes vipera* [22]. These serine proteases have different molecular weight (sizes), however, currently there is no structural report of the enzyme and thus yet to be focused to reveal their functional mechanism on the coagulation factors.

Prothrombin Activator-SP

A large number of venomous snakes contain prothrombin activator (PA) protein which stimulates the coagulation process by forming the insoluble fibrin in the blood clotting cascade [24, 25]. Depend on the concentration and specificity (turn over) of the SP, the clotting/prothrombin time is determined. The prothrombin activator is classified into four groups based on the structural fold, mechanism of action and cofactors [26, 27]. Groups A and B (Metalloproteases-converts thrombin to meizothrombin) and groups C and D (Serine proteases-converts prothrombin to mature thrombin).

The group of prothrombin activator-C (PTA-C) awfully needed only Ca 2+ ions and negatively charged phospholipids not factor Va, for the prothrombin activation process. The PTA was reported from the venom of *Oxyuranus scutellatus* [28] and *Pseudonaja textilis [29]*. Native form of PTA-C consisting of a factor Xa-like catalytic subunit (60 kDa) and a factor Va-like cofactor subunit (200 kDa). The two chains of factor Xa-like catalytic subunit are linked by several disulfide bridges, while the two chains of the factor Va-like subunit is held together by the strong non-covalent interactions. The catalytic subunit (factor Xa-like domain) have weak catalytic function but it's stimulated by the factor Va-like subunit in the enzyme [30, 31]. These activators hydrolyze the prothrombin at the positions of the peptide bonds, Arg 271-Thr 272 and Arg320-Ile321, and produces the active and mature thrombin. Also, it shown that the group C prothrombin activators structurally and functionally resemble the mammalian factor Xa-Va complex [32, 33]. In contrast, groups A and B activators convert prothrombin to Meizothrombin, a protease precursor of thrombin, is cleaved at single position and it's an intermediate to mature thrombin.

The functional activities of prothrombin activator-D (PTA-D) is strongly stimulated by Ca^{2+} ions, factor-Va and negative charge phospholipid vesicles [34-36]. The PTA-D have two chains (light and heavy chains) adhered together by the disulfide bridges formed between them and the active site is located in the heavy chain. They exhibited an efficient procoagulant effects through the activation of prothrombin in comparison to the mammalian factor-Xa [37]. Similar to factor-Va, the factor Xaa also cleaves the prothrombin at the

same sites, Arg271 -Thr272 and Arg320-Ile321. The activator activity is increased to a million-fold by the involvement of the negative charge phospholipids, factor-Va and Ca^{2+} ions. The amino acid sequences of trocarin-D and hopsarin-D are derived and they share high identity (53-60%) and similarity (62-70%) thus forms the similar kind of domain structure with factor Xa. The light chains of the activator consist of N-terminal Gla-domain (residues 1-39), two EGF-I (Epidermal growth factor-like domain, residues 50-81), and EGF-II (residues 89-124). And finally it shows that the group-D venom prothrombin activators are have significance structural and functional homologue of blood coagulation factors [38, 39].

Thrombin-Like Enzyme-SP

Thrombin-like enzymes (TLE) are found in several pit viper genera (*Agkistrodon*, *Bothrops*, *Crotalus*, *Lachesis* and *Trimeresurus*), common vipers (*Bitis* and *Cerastes*) and the colubrid, *Dispholidus typus* [40-42]. TLEs are single-chain serine proteases (33), except the enzyme from *Cerastes cerastes*, which consists of two identical disulfide-linked subunits (MW ~26-33 kDa) with some glycoproteins [43]. It shares 67% sequence identity between them and 40% with the human thrombin. The conservation of Asp189 found in all the TLEs shows that the cleavage of fibrinogen is more specific for the serine protease thus hydrolyze the peptide bond (arginyl bond) after basic residues [40]. The main difference is that TLEs prefer to release either fibrinopeptide A or B with equal efficiency, unlike the thrombin which produces both the peptides. The low molecular weight serine protease inhibitors are inhibiting the TLEs, but most of the molecules are not inhibited by the thrombin inhibitors like anti-thrombin III and hirudin [41]. They act on blood plasma and induce powder type and translucent clots due to the lack of cross-link between the fibrin by the action of factor-XIIIa. TLEs often act on coagulation factor-XIII but appears to be degrade it rather than the activation. Thrombin has wide role in the activation of the coagulation whereas TLE has no effect on the other coagulation factors [44]. Though TLEs resembles to thrombin, their structure and function is distinctive to the coagulation factor [40, 45]. The unique property of TLEs empower the clinical use as the defibrinogenating agents; like, ancrod (Arvin; from *Calloselasma rhodostoma*) and batroxobin (Defibrase; from *Bothrops atrox moojeni*) [46, 47]. The fibrin formation is not favoured by the cross-linking, hence it can be degraded by the fibrinolytic process. So, the TLEs are clinically very well tolerated with no or less side effects.

Anticoagulant -SP

Anticoagulants from snake venoms are involved in the prolong clot formation; they are enzymes, such as Serine Proteases and Metalloproteases, or nonenzymatic proteins, such as C-type lectin and three-finger toxins.

Protein C is an anticoagulant protein, involves in important mechanism of blood coagulation process. The anticoagulation protein C regulates blood coagulation pathway by inactivating factors V/Va and VIII/VIIIa through increasing the fibrinolytic activity. Protein C is activated (APC; activated protein C) by thrombin and thrombomodulin complex then the APC forms complex with protein S (PS; cofactor for APC), thus degrades FVa and FVIIIa and leads to the loss of the factors activities. Many snakes venom have specific molecules which activates the protein C an important key molecule in the blood coagulation [20, 48]. Venom of the snake species like Agkistrodon genus (*A. contortrix, A. c. mokasen, A. c. pictigaster, A. piscivorus, A. p. leucostoma, A. halys halys, A. blomhoffi ussuriensis and A. bilineatus*) and *Bothrops moojeni, B. pradoi, Vipera lebetina, Daboia russelli, Cerastes cerastes, Trimeresurus mucrosquamatus* contain the protein C activators and they are characterized but further studies has to be carried for the similar and distinct function of the activators. The protein C activators are glycoproteins (MW ~36-40 kDa) the SP activates protein C at the lower salt concentration in absence of Ca^{2+} ions, in contrast at higher salt and in presence of Ca^{2+} ions the ability of activation of protein C is reduced [49, 50]. The Protein C activators are used as diagnostic agents in the estimation of protein C [51, 52].

The commercial product, Protac@ has been developed from purified the fast-acting protein C activator from *A. contortrix contortrix*. Protac converts the zymogen protein C into an active serine proteinase hasten and independent of thrombomodulin which causes both enzymatic and anticoagulant effects. Protac also activates protein C from cattle, sheep, goat, rabbit, horse and pig. The active component of Protac is a protease which causes the activation of PC and is used for the determination of PC (Protein C Disorder), PS (Protein S Disorder), APC-Resistance and global assays for detecting of protein C pathway disorders. The PC activators function from *A. contortrix contortrix* venom have been determined in a relative short time and the protein C activator is utilized widely as a diagnostic tool in research, medicine and clinical usages [51].

Fibrinolytic -SP

The blood coagulation cascade and fibrinolysis are coordinated by special regulatory molecules like cofactors, inhibitors, and receptors [53]. Plasmin is the primary fibrinolysin, activated by Plasminogen activator (PA), a serine proteases (tPA or uPA) from the zymogen (plasminogen). Both the activators have short life span in the blood circulation (between 4-8 minutes) due to the presence

of high concentration of inhibitors like plasminogen activator inhibitor-1 (PAI-1). The proteases of snake venom contribute a large for fibrinolysis by the activation of plasminogen to plasmin and reports show that some Metalloproteases also have the fibrinolytic activity [54].

Plasminogen activators have been purified from the venoms of *Trimeresurus stejnegeri* (TSV-PA), *Agkistrodon halys* (Halys-PA) [55] and *Lachesis muta muta* (LV-PA) [56]. These serine proteases are acidic glycoproteins (MW ~33 kDa). TSV-PA activates the plasminogen by a single cleavage at the peptide bond, Arg 561 -Val 562 110[57]. The structure-function study of TSV-PA have shown that Phe193 plays an important role for plasminogen binding and inhibitors too. The mutation study shown that the replacement of Phe193 by Gly resulting 8 to 9 fold increase in the enzyme activity on plasminogen [58] where the mutant is highly susceptible (100-fold) to PAI-1 and 2 -antiplasmin [59]. In addition, 37-loop of TSV-PA plays a crucial role in the resistance to the inhibition by the protease inhibitors [60]. The snake venom serine proteases are largely involved in the blood coagulation and fibrinolytic process and less in the other biological functions and they are one of the important toxin protein for the therapeutic drug development.

3-Finger toxin

Previously, three-finger toxins (3FTxs) are known to be specific to elapidae venoms, later a-colubritoxin [61], venom protein characterization by Pawlak et al. 2006, 2009; Heyborne and Mackessy, 2013 and transcriptome and proteome analysis by Junqueira-de-Azevedo et al. 2006; Pahari et al 2007; McGivern et al. 2014; Modahl et al. 2016 and reveals that 3FTxs are widely present in diverse snake species.

Various 3FTx receptors have been identified as the interacting molecules with L-type calcium channels, integrins [62], nicotinic and muscarinic acetylcholine receptors [63], coagulation factor VIIa [64], and β1/β2-adrenergic receptors [65]. Venom 3FTxs also exhibit diverse biological activities viz. neurotoxicity, cardiotoxicity, cytotoxicity, and anticoagulation effects [66](Hegde et al. 2010). An elapidae post-synaptic α-neurotoxin tightly binds to the nicotinic acetylcholine receptor and results in the rapid blockage of the ion channel which leads to deliberated paralysis activity of prey [67]. In comparison to the rear-fanged venom 3FTxs, α-cobratoxin isolated from *Naja kaouthia* (front-fanged) venom has been shown that it is equally toxic to both lizard and mouse animal models [68].

Non-toxic Venom Proteins

Disintegrins

Disintegrins represent an interesting class of low MW, RGD-containing (Arg-Gly-Asp) and cysteine-rich peptides characterized from different snake venoms. They are interact with integrins, family of protein which constitutes of α and β heteromeric subunits helps to make the cell-extra cellular adhesion. Disintegrins preferred to bind with β1 or β2 containing integrins, prevents the interaction with the natural ligands which regulates the cellular transduction and they represent the potent inhibitor molecule for some integrins [69, 70].

Disintegrins, small polypeptide (range 40-100 AA) are act as robust inhibitors for the platelet fibrinogen receptor, integrin-IIb/IIIa [71]. Some disintegrins (only dimeric disintegrins with non-RGD sequence) not able to inhibit the platelet aggregation [72, 73]. The disintegrins were classified into five different groups by concerning the number of disulfide bonds and length of the polypeptide chain [74]. The first, is short disintegrins (41-51 AA) with four disulfide bonds, second, the medium-sized disintegrins (about 70 AA) with six disulfide bonds, third, the long disintegrins (nearly of 84 AA) with seven disulfide bonds and fourth, where disintegrin-like domains was derived from PIII-SVMPs thus the disintegrins are proteins combined with a N-terminal disintegrin-like domain (~ 100 AA) with eight disulfide bonds and a C-terminal (110-120 AA, cysteine-rich domain) with six disulfides units [75]. Finally, the fifth is established by homo or heterodimers where the dimeric disintegrins are contain two subunits (with 67 residues) formed by four intra and two inter chain disulfide linkages [76, 77]. Bilitoxin-1 is an example of homo-dimeric disintegrin comprised of disulfide links where each unit contains of 15 cysteinyl residues [77]. The non-enzymatic proteins, disintegrins are commonly found in the venom of viperidae [78] and the function is more specific and selective for the blocking of the integrin receptors which is present in the cell membranes [79, 80].

Distegrins are specific molecules for the platelet glycoprotein receptor IIb/IIIa and they are one of the persuasive competitive inhibitors for fibrinogen dependent platelet aggregation which is induced by the key molecules thrombin, collagen, ADP and epinephrine [81-83]. The specific binding of disintegrins (echistatin and kistrin) favour the inhibition of integrin at very low concentration (nanomolar) in some animal models. There more advantage for the therapeutic usage of disintegrins for the inhibition of platelet aggregation in vivo due to its short life span and non-toxic property [81]. Currently, Echistatin is used as an effective inhibitor at the nanomolar concentration for the bone resorption in the cell culture environment [84].

C-type Lectin

C-type lectin-like proteins (CTLs) are largely present in snake venoms and responsible for various functions, especially hemostasis, blood coagulation and platelet activation processes. CTL proteins have 15–40 % sequence homology with the carbohydrate recognition domains of the C-type lectins [85]. They are belongs to non-enzymatic, Ca^{2+}-dependent ("C") proteins which prefers to bind with the sugar residues [86]. On the other hand, most of CTLs not able to bind with Ca^{2+} thus leads to loss of the interaction with carbohydrates (galactose) [87]. The CTLs are heterodimers composed of homologous α (MW ~14-15 kDA) and β (MW ~13-14 kDa) subunits structures. The combination of covalent and non-covalent type of multimerization of the heterodimer gives α/β, (α/β)2 and (α/β)4 CTL structures. In contrast, conventional C-type lectins form homodimers which are linked by only inter-chain disulfide bridges [88]. Heterodimers composed of α/β of CTLs are formed by domain-swapping where domain from the one subunit replaces the appropriate domain of the other subunit.

CTLs play an important biological roles in endocytosis, adhesion, and pathogen neutralization [89]. The targeted functions of CTLs are membrane receptors, coagulation factors, and protein factors essential to hemostasis. For platelet activation and aggregation processes, the adhesion receptor molecules of platelets viz. vWF (von Willebrand factor) binding GPIb-complex, collagen binding GPVI and integrin α2β1, and fibrinogen receptor integrin aIIbβ3 are playing vital role and the CTLs also interacting with these molecules. Some CTLs act as antithrombin and others are function as anticoagulants by the inhibition of the coagulation factors, driving to the hemorrhages, while other CTLs activate coagulation factors without the need of any cofactors [90]. Lectins from the snake venom Galactose-specific C-type lectins have been characterized from the venom of the rattlesnake *Crotalus atrox* (Hirabayashi et al., 1991), *B. jararaca* (Ozeki et al., 1994), and other snakes belonging to the Elapidae and Viperidae.

The structures of CTLs, contain a CRD motif, and belong to the group VII C-type lectin family, they show diverse pharmacological activities against coagulation factors and platelets and affect hemostasis and thrombosis. They can be classified into four subgroups on the basis of their functions, namely lectins, coagulant proteins, platelet aggregation agonists, and platelet aggregation antagonists. They are namely, (i) Lectins (Homodimer, PDB ID: 1MUQ) bind to galactose (sugar molecule), (ii) Anticoagulant protein (Homodimer of α/β subunits; PDB ID: 1IXX, 1BJ3) interacts with factor IX/X, (iii) platelet aggregation agonist (PDB ID: 1UMR, 1FVU, 1IJK) protein regulates GPIa/IIa, GPVI, GPIb, vWF(GPIb) and (iv) platelet aggregation antagonists (PDB ID: 1C3A, 1UKM) have interaction with GPIb, GPIa/IIa. As the structural and functional evolution of the protein continues, the understanding of the diversity and specificity of CTLs are keep on flourishing.

Hyaluronidase – "Spreading Factor"

Hyaluronidases (EC:3.2.1.35) are class of glycoside hydrolases family enzyme (carbohydrate-active enzyme), involved in the carbohydrate metabolism particularly with Hyaluronan (HA), a GAG molecule (glycoaminoglycans). There is a need of more studies to unravel the mechanism and suspicions about Hyaluronidase functions and specificity, hence they are the category of the neglected type of enzymes [91, 92]. Hyaluronidase hydrolyze the substrate HA at β(1–4) bond between N-acetyl glucosamine (NAG) and glucuronic acid (GUA). The cleavage is supported by two residues where one acts as generic acid (a proton donor) while other acts as a base/nucleophile [93]. Though, all animals consists of Hyaluronidase, venom proteins contain functionally diverged Hyaluronidase and they are very active in all the secreted type of venoms. Biochemical characterization of Hyaluronidase have been reported widely in the venom components of bees, spiders, scorpions, hornets, stone fish and many snakes [94-99]10,13–17. The venom of *Apis mellifera* (honey bee) contains the Hyaluronidase (a major allergen of bee venom) that induces life threatening anaphylactic reactions in humans [100].

Our recent study shown the sequence diversity of viper family Hyaluronidase and found that the E-loop of viper (*Echis Carinatus*) might have significant for the fast venom spreading and we have reported that Myricetin or its derivative compounds would be a prominent inhibitor for the venom spreading [101]. The cancer growth is induced by human Hyaluronidase-1 so the drug molecule can be able to control the cancer progression and angiogenesis but further experimental support has to be conducted. More functional studies have to be conducted to explore the understanding and mysteries of venom Hyaluronidases.

CRISP (Cysteine-rich secretory proteins)

Cysteine-rich secretory proteins (CRISPs) are a family of non-enzymatic proteins which is widely distributed in the venom proteins. CRISPs are have high degree of conservation in the sequence and the structure and sixteen highly conserved cysteine forms eight disulfide bridges. CRISPs have diverse biological functions like binding with cyclic nucleotide-gated ion channels [102], inhibits the vascular smooth muscle contraction [103], inhibiting the Ca^{2+} ion release from SPR (SarcoPlasmic Reticulum) [104], and blockade of the calcium currents in neurons [105].

The isolation and characterization of CRISPs have been reported from the venoms of Elapidae Viperidae and

Colubridae [103, 106], as well as from the venom of Mexican beaded lizard (*Heloderma horridum horridum*; [107]). An evaluation study of the CRISP molecule commenced that they are under gone a strong positive selection in the snakes than in the lizards [108]. Patagonin (MW of 24.8 kDa), a CRISP was isolated from the venom of *P. patagoniensis*, exhibits a unique necrotic activity towards the murine gastrocnemius muscle at higher doses and does not have edema, hemorrhage, and aggregation of human platelet effects [109] along with the proteolytic activity towards the azocoll, azocasein, or fibrinogen assays.

Therapeutic Drugs from Venom

There are many drugs (peptide/protein) from the animal venom which is highly specific and effective for the particular disorder or diseases. And, more studies have been conducting to screen the new drug molecules from the animal venoms. Here is the list of approved and testing drugs in clinical trials (in 2016 onwards).

Table 1: List of Approved and Clinical Trial Drugs

Drug Name	Name of organism	Mechanism of action	Medical Application
Captopril (CAPOTEN)	Jararaca pit viper (*Bothrops jararaca*)	Angiotensin-converting enzyme inhibitor	Hypertension and cardiac failure
Enalapril (VASOTEC)	Jararaca pit viper (*Bothrops jararaca*)	Angiotensin-converting enzyme inhibitor	Hypertension and cardiac failure
Exenatide (BYETTA)	Gila monster lizard (*Heloderma suspectum*)	Glucagon-like peptide-1 receptor agonist	Type 2 diabetes mellitus
Ziconotide (PRIALT)	Magical cone marine snail (*Conus magus*)	$Ca_v2.2$ (Calcium) channel antagonist	Management of severe pain
Bivalirudin (ANGIOMAX)	Europian medicinal leech (*Hirudo medicinalis*)	Reversible dierect thrombin inhibitor	Anticoagulant in percutaneous coronary intervention
Lepirudin (REFLUDAN)	Europian medicinal leech (*Hirudo medicinalis*)	Binds irreversibly to thrombin	Anticoagulation in heparin-associated thrombocytopenia; thromboembolic disease
Desirudin (IPRIVASK)	Europian medicinal leech (*Hirudo medicinalis*)	Selective and near-irreversible inhibitor of thrombin	Prevention of venous thrombotic events
Tirofiban (AGGRASTAT)	Saw-scaled viper snake (*Echis carinatus*)	Antagonist of fibrinogen binding to the GPIIb/IIIa receptor	Acute coronary syndrome
Eptifibatide (INTEGRILIN)	Pigmy rattlesnake (*Sistrurus miliarius*)	Prevents binding of fibrinogen, von Willebrand factor, and other adhesive ligands to GPIIb/IIIa	Acute coronary syndrome percutaneous coronary intervention
Batroxobin (DEFIBRASE)	Common lancehead snake (*Bothrops atrox*) or Brazillian lancehead snake (*Bothrops moojeni*)	Cleaves Aα-chain of fibrinogen	Acute cerebral infarction; unspecific angina pectoris; sudden deafness
Drug Name	**Name of organism**	**Mechanism of action**	**Medical Application**
Platelet gel (PLATELTEX-ACT)	Common lancehead snake (*Bothrops atrox*)	Cleaves Aα-chain of fibrinogen	Gelification of blood for topical applications
Fibrin sealant (VIVOSTAT)	Brazilian lancehead snake (*Bothrops moojeni*)	Cleaves Aα-chain of fibrinogen	Autologous fibrin sealant in surgery
Thrombin-like enzymes	Chinese moccasin snake (*Deinagkistrodon acutus*) or Siberian pit viper	Fibrinogenase	Antithrombotic; defibrinating agent for the treatment and prevention of thromboembolic disease

	snake (*Gloydius halys*) or Ussuri mamushi viper (*Gloydius ussuriensis*)		
Hemocoagulase (REPTILASE)	Common lancehead snake (*Bothrops atrox*) or Jararaca pit viper snake (*Bothrops jararaca*) or Brazilian lancehead snake (*Bothrops moojeni*)	cleaves Aα-chain of fibrinogen; factor X and/or prothrombin activation	prophylaxis and treatment of hemorrhage in surgery
Medicinal leech therapy	European medicinal leech (*Hirudo verbana*) or other species	inhibits platelet aggregation and the coagulation cascade	skin grafts and reattachment surgery
Dendroaspis natriuretic peptide (cenderitide, CD-NP) Capricor Therapeutics	Eastern green mamba snake (*Dendroaspis angusticeps*)	inhibit platelet aggregation and the coagulation cascade	Congestive cardiac arrest (UCT-2016)
Stichodactyla toxin ShK (dalazatide, ShK-186) Kineta	Sun sea anemone (Stichodactyla helianthus)	$K_v1.3$ channel antagonist	psoriatic arthritis, multiple sclerosis, lupus, rheumatoid arthritis, and other autoimmune diseases (UCT-2016)
Chlorotoxin (BLZ-100, TM-601) Blaze Bioscience	Deathstalker (Yellow scorpion) (Leiurus quinquestriatus)	binds to membrane type-1 matrix metalloproteinase (MMP), MMP-2 matrix MMP-2, tissue inhibitor of metalloproteinase-2, CLC-3 Cl- channel in glioma cells and other tumors of neuro ectodermal origin	glioma and other central nervous system tumors (UCT-2016)
Soricidin (SOR-C13) Soricimed	Northern short-tailed shrew (Blarina brevicauda)	inhibitor of the Ca^{2+}-selective transient receptor potential channel TRPV6	Ovarian and other cancers (UCT-2016)

Here, UCT means "under clinical trials", for further details visit the website: http://zoltantakacs.com/venom_medicines_snake_toxin_drugs_zoltan_takacs.shtml

Conclusion

The venom proteins composition (concentration) and the protein/molecular type determines the toxicity of the animal venom and diversity of each protein is very high, hence the specific function and molecular character has to be studied explicitly. One aspect the animal venom is more dangerous for the victims including human (as millions of people are affected and dying by snake bite and other venom stings). In other aspects they are beneficial and curing lots of medical complications if we administrate the specific molecule as a drug. The knowledge of animal venom to human is less understood and large has to be explored to find many drugs to the large number of health issues including cancer. There is more than 20 million of toxins remains

unexplored from over 1,00,000 venomous animals in the world. The large molecular biodiversity of animal venoms has extensive treasured for the development of novel pharmaceutical drugs for the current health complications [110]. The deadly poisons from the animal venoms can be converted into fascinating lifesaving drugs with understanding the molecular properties and successful clinical trials.

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