Chapter

Snake Venom

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Abstract

Venomous snakes belonging to the family Viperidae, Elapidae, Colubridae and Hydrophidae, produces snake venom in order to facilitate immobilization and digestion of prey, act as defense mechanism against threats. Venom contains zootoxins which is a highly modified saliva that is either injected *via* fangs during a bite or spitted. The modified parotid gland, encapsulated in a muscular sheath, present on each side of the head, below and behind the eye, have large alveoli which temporarily stores the secreted venom and later conveyed by a duct to tubular fangs through which venom is injected. Venoms are complex mixtures of more than 20 different compounds, mostly proteins and polypeptides, including proteins, enzymes and substances with lethal toxicity which are either neurotoxic or haemotoxic in action and exert effects on nervous/muscular impulses and blood components. Lots of research are directed to use venoms as important pharmacological molecules for treating various diseases like Alzheimer's disease, Parkinson's disease etc.

Keywords: snake, venom, toxin, proteins, haemotoxic, neurotoxic, pharmacological molecules

1. Introduction

Snakes produce venoms that finds its use in immobilizing and digesting the prey and acts as an effective defense system against threats. Thus, venom is a functional trait utilized by an organism to regulate homeostatic processes of another organism i.e., it mediates the outcome of interactions between two or more organisms [1]. These snake venoms are storehouse of fascinating and useful bioactive compounds. Although various studies have been carried out on venoms, only few of them are well understood and tapped for their potential use in medicine as pharmacological molecules and diagnostics, understanding the molecular mechanisms of bodily processes such as homeostasis, coagulation, thrombosis, angiogenesis, and metastasis. The enthusiasm to understand animal envenomation and associated medical treatments has driven the animal venom studies in a multifaceted manner [2]. This chapter deals with what is venom, need for venom, evolution of venom, the poison apparatus that aids in producing the venom, genetics and biochemistry of the venom, effects of venom, antivenom, and applications of venom as therapeutics, diagnostics, and as biochemical tool.

2. Venom

Venom in layman's terms is modified snake saliva. This concoction is a combination of zootoxins, enzymes, and pharmacologically active peptides [3–5].

Digestive enzymes and other proteins that act as a paralyzing and pre-digestive agent. Digestion is therefore initiated outside the predator's alimentary canal while simultaneously immobilizing the prey. The snake then swallows the prey whole, liquefying most of the tissue and discarding what cannot be digested (feathers/hair/claws) along with the fecal matter. Although venomous snakes apply venom in the acquisition of the prey, they also fan out them in defensive bites against intimidating predators and aggressors.

2.1 The need for venom

Snakes are carnivores and actively hunt their prey. Most of them are ambush hunters and generalized feeders. Their geographic distribution and dietary preferences being varied, snakes had to evolve a method of incapacitating their prey quickly and their answer was venom. Geographic location and varied diet have led to the development of more and more complex venom. Being generalized feeders, many species of snake have a larger repertoire of proteins in their venom that affect individual prey animal species differently [6]. Honing the composition, mechanism of delivery, dosage and action of venom remains one of nature's greater success stories to date. Venom production has aided the proliferation and diversification of snakes as a group.

2.2 Evolution of venom in snakes

The evolution of snakes remains a partially solved mystery to scientists. Though they have known that snakes are descended from lizards since the 1970's there are many missing links due to a lack of proper fossil evidence. Debates of their descent from aquatic or terrestrial lizards persist as there is evidence to support both hypotheses. But the evolution of venom on the other hand has started to unravel. Initial research suggested that the venom and the venom delivery apparatus evolved together. But in 2002 a group of scientists in Australia led by Dr. B. Fry made amazing roads into the history of the evolution of venom. The major factor in the previous theory was that though many species had fangs they were in different locations in the jaw, size, and structure. The venom gland on the other hand remained the same.

Dr. Fry and his team examined the idea that the production of venom must predate the use of fangs. They looked at the extant lizard species which were said to contain toxins in their saliva. They discovered that the Komodo dragon and many of their related monitor lizards all had venom glands at the base of the lower jaw. This venom mixed with saliva caused damage to the prey previously thought to be caused by bacteria mixed in the saliva. The venom was like snake venom [7]. Most lizard species have been shown to have a gland like the venom gland of snakes, this led to the theory that snakes and lizards had a common venomous ancestor and that venom predated fangs.

Discoveries have shown around 1500 species of lizards had components like snake venom. Sea snakes are considered to have diverged from terrestrial snakes around 30 mya. Their venom is highly toxic. Studies have shown that they are evolving more complex toxin molecules to suit their ever-changing prey. This goes to show that venom is still evolving. The dietary preferences of the snake can magnify the change in venom. The snake can produce a potent mixture that can affect different prey animals uniquely [6]. There is no efficient antivenin generated against sea snakes.

There are so many species of snakes whose venom composition is yet to be studied in detail. These studies are crucial in understanding the evolution of venom and the effect of venom on prey species [8].

3. Poison apparatus

In reptiles, twice the venom glands have evolved; once in helodermatid lizards and secondly in advanced snakes belonging to colubroids, viperids, elapids and astractaspidids [9]. Venomous snakes belong to 4 genera. These snakes possess a poison apparatus or venom producing glands in their heads, which produces toxic substance that acts as either a poison or a venom. When the toxic substance is injected into the body of prey, it is venomous.

3.1 General plan

A poison apparatus of a snake consists of snakes consists of 4 major parts, namely, a pair of poison gland, poison ducts, fangs and muscles.

3.1.1 Poison glands

These glands situated on either side of the upper jaw, is possibly the superior labial glands or parotid glands. Each poison gland has a sac-like capsule and a narrow duct at the anterior end. The vascular fibrous septum of the capsule separates glandular substances into secretory pockets. The duct after passing along the sides of the upper jaw, opens at the base of the fang or at the base of the tunnel on the fang. The poison glands are held in position via anterior and posterior ligaments, which attaches anterior end of glands to maxilla and posterior end to the quadrate respectively. The fan shapes ligaments are situated between the side walls and squamosal-quadrate junction.

3.1.2 Ducts

The pair of ducts opens into a pocket of mucous sheath that covers the basal part of the fang. In spitting cobras (*Naja nigricollis*), the poison duct is modified into 'L' shaped bend prior to exiting the fang.

3.1.3 Fangs

The fangs evolved to inject venom into the prey is a grooved or tubular tooth. The paired pointed and hook-like teeth are modified form of maxillary teeth. They are long, curved, sharp and pointed. Based on the structure and position, fangs are of 3 types:

i. Proteroglyphous (Protero – first)
These are small, grooved, articulated at permanently erect at the anterior end of maxillae. They are found in Cobras, Kraits, Coral snakes and Sea snakes.

ii. Opisthoglyphous (Opistho - behind)
They are small and grooved but remain associated with posterior end of maxillae.

iii. Solenoglyphous (Solen – pipe; glyph - hollow)
This type of fangs is seen in vipers and rattle snakes. A large functional fang occurs on the front of each maxilla and are movable and turned inside to lie in the roof of mouth when it is closed. This fang contains a narrow hollow poison canal with enamel, which opens at anterior end of the fang.

3.1.4 Muscles

Positioning and functioning of the poisonous apparatus is enabled by the presence of 3 types of muscle bands, namely, Digastrics, Sphenopterygoid, and Anterior and posterior temporalis.

3.2 Venom glands of elapidae

Early description about the elapid venom gland dates to 1936 [10]. *Elapidae* venom gland is enclosed in a tough capsule of connective tissue and more compactly built than that of viperid snakes. It consists of a posterior main gland and an anterior secretory duct with an accessory mucous gland. Simple or compound multiple contiguous tubules that run in a posterior–anterior direction is seen in the main gland. The tubules converge toward the centre of the gland and open into a small lumen. The secretory epithelium is of a serous nature. Secretory cells of elapids at resting stage are loaded with granules that differ in structure and number than those found in viperid venom gland. The cells of the accessory glands are PAS-positive, and their secretions mainly consists of sialomucins [11].

3.3 Venom glands of viperidae

The venom glands belonging to two viperid subfamilies, namely *Viperinae* and *Crotalinae* exhibit similarity in shape and structure. Except for the mole vipers belonging to the genus *Atractaspis*, all other have a glandular structure. The mole vipers differ in not possessing a differentiated accessory glands unique to the "genuine" vipers. The glands consist of large numbers of radial tubules surrounding a central lumen. The tubules are unbranched, and the luminal end consists of a mucous epithelium [12]. The venom of *Atractaspis engaddensis* has a relatively high alkaline monophaosphatase activity and is devoid of arginine ester hydrolase activity that is seen in other vipers. The first person to give a detailed account of venom glands of a true viper, *Vipera berus,* is Wolter in 1924 [13].

The venom gland has four distinct regions: the main gland, the primary duct, the accessory glands, and the secondary duct that leads to the fang sheath. The accessory glands have two distinct regions. The anterior part is lined by mucous epithelium that contains goblet cells while the posterior part is lined with flat to cuboidal epithelium, correlating with the secretory function [14]. The main gland is made of repeatedly branched tubules arranged around a large central lumen, where a considerable amount of venom can be stored. The tubules are made of secretory cells.

3.4 Venom glands of colubroidea

A pair of homologous oral venom glands located behind the eye on either side of the upper jaw are connected to the ducts that transfers the secreted venom to the base if morphologically diverse teeth, fangs [15].

3.5 Venom glands of sea snakes

The venom glands and related muscles of sea snakes are like the general structure that we observe in the terrestrial elapids. The considerable reduction in venom gland as well as the accessory gland is attributed to the aquatic environment. An early divergence of sea snakes from an ancestral elapis stock has been proposed as the musculus compressor glandulae is well developed in the sea snakes. A possible

phylogenetic relationship exists between Australian elapids and hydrophiine snakes which is evident from the similarities that exists between them [16].

3.6 Changes in venom gland following milking process

Morphological changes in the secretory epithelium of venom gland after the expulsion of venom was noticed by Velikii in *Vipera ammodytes* [17] which was later confirmed by further studies [18, 19].

4. Genetics of snake venom

The bioactivity of the venom is determined by the complex and variable interactions between genes, their expression, their translation, and their post translational modification. Evidence that the loss of genes also has a strong influence on shaping venom phenotypes further reinforces the usage of animal venom systems to understand adaptation in the natural world is evident from the loss of genes that have a strong effect on forming the venom phenotype [20].

5. Biochemistry and physiology of snake venom

Venom was identified to be a proteinaceous concoction in the 1800s. 90 to 95% of the dry weight of venom is made of proteins. These proteins are also responsible for the biological effects of the venom. These proteins can be classified as enzymes and toxins [21]. The components of venom can vary from animal to animal within a species too. Research has shown that age [22], gender [23], geographic location [24], prey species/diet [25] and season [26] can all influence the composition of venom. All the proteins involved in venom are repurposed from regular physiological functions.

The proteins identified in venom have been studied individually, as protein complexes and as protein families. The proteins in the complex can be homodimers (made up of identical subunits) or heterodimers (made up of different subunits – sometimes these subunits are from different families). These complexes are held together by covalent bonds and the complexes are pharmacologically more potent than the individual enzymes or proteins. The complexes seem to expose critical residues that otherwise may have been buried in the individual enzymes [27].

5.1 Enzymes in snake venom

Typically, snake venom contains hundreds of components, all of which work in tandem to paralyze the prey and initiate digestion. Many enzymes are found and even some toxins have enzymatic functions. The most studied enzymes and their role are discussed below.

5.1.1 5' Nucleotidase (5'-NT)

This is an enzyme made up of 548 amino acids and a molecular mass of 61 kDa found in almost all living cells. The enzyme hydrolyses nucleosides. It is found in all snake venom around the world. Isoforms have also been isolated, like the isoform from the venom of *Vipera lebetina* (Cypriot blunt-nosed viper) that is found to be a homodimeric monomer with a molecular mass of 60 kDa. This isomer inhibits ADP- or Collagen-induced platelet aggregation [28]. The isomer isolated from the

Japanese pit viper (*Gloydius blomhoffii blomhoffii*) shows that the enzyme has 2 binding sites for Zn + and can exist as a trimer or a tetramer [29]. Venomous snakes belonging to the family Viperidae (*Vipera russelli russelli*- Russell's viper, *Echis carinatus*- Indian saw-scaled viper, *Eristocophis macmahonii* - Asian sand viper) were studied in the country of Pakistan and were all found to have high 5'-NT activity venom wise [30]. Among the snakes in Brazil that were studied, *Bothrops brazili* (Brazil's lancehead) had the highest 5'-NT activity. On the other hand, venom from *Philodryas olferssi* (South American green racer), a snake endemic to South America, showed little or no activity of 5'-NT. Among all the snakes studied there were differences in zymology and banding patterns among the enzymes thereby implying important physical structural differences [31]. The enzyme 5'-NT is found to act synergistically with other enzymes and have a pronounced anti-coagulant effect. It is said to liberate adenosine, and this helps immobilize the prey. In 2008, it was showed that the whole enzyme or a part of it is secreted in the venom. The soluble form of the enzyme is released by cleavage of the ectodomain in the venom gland or specialized tissues [29].

5.1.2 Acetylcholinesterase (AChE)

It is the primary enzyme that catalyzes the breakdown of Acetylcholine in the body among other related neurotransmitters. AChE is found in nerve and muscle tissue, especially abundant in synaptic junctions. This is perhaps one of the well-studied enzymes from snake venom, its structure has been elucidated in detail. AChE is abundant in the venom of all snakes and higher concentrations are observed in the Elapid snakes except the Mambas [32]. Although the enzyme is present in the venom of snakes belonging to Viperidae and Crotalidae the activity was not detected. The highest concentration of venom AChE (VAChE) is found in the venom of *Bungarus* sp. 8 mg/gm of dried weight [33]. VAChE is found to be optimally active at 45° C and pH 8.5 [34].

The protein structure of VAChE shows homology to mammalian and Torpedo AChE with a few major changes. These changes ensure that VAChE has a less complicated structure than membrane-bound AChE. Many isoenzymes exist and can be differentiated on charge alone [35]. Protein structure has been studied from the VAChE of *Bungarus fasciatus* (BfAChE) and *Naja naja oxiana* (NnAChE). BfAChE exists as a soluble hydrophobic monomer. The C terminal peptide has an alternative exon ('S'). It is made up of 15 residues, the last 8 of these are removed in the mature protein. Compared to mammalian AChE, BfAChE has the following changes: Tyr70 is replaced by Met70, Acidic residue285 (glutamate/aspartate) is replaced by Lys285. The active site gorge is 20 A° deep and has two ligand-binding sites [36]. The crystallized structure shows evidence for a co-existing open/closed state in the back door channel and semi occuled gorge entrance. The presence of Met70 enlarges the entrance of the gorge, enabling better binding. It can form canonical dimers of subunits despite non-amphiphilic C terminus.

The NnAChE exists as a monomer at 0.2 mg/ml and a dimer at 2 mg/ml. It is a single polypeptide chain with a molecular weight of 67,000 ± 2000 Da and exists in several isoforms with different isoelectric points [37]. It differs from BfAChE by having a dimerization domain where His replaces Pro at position 514 [38]. VAChE has been associated with acute neuromuscular paralysis and neuromuscular weakness. This may be due to a defective transmission in the neuromuscular junction [5]. The function of AChE in elapid venoms could be to aid in the immediate hydrolysis of acetylcholine released from synaptic vesicles. This release could be under the influence of β-neurotoxin to avoid competitive protection by acetylcholine of postjunctional receptors against α-neurotoxin [39].

5.1.3 Phosphatases–acid phosphatase (ACP) and alkaline phosphatase (ALP)

These enzymes are found in lysosomes and during digestion they work on releasing the phosphoryl groups from molecules. They are found in all snake venoms. Both enzymes have a greater action in Elapids than Viperids. In a study that compared *Cerastes cerastes* (Saharan horned viper), *Cerestes vipers* (Saharan sand viper), *Naja haje* (Egyptian cobra) and *N. nigricollis* (Blacked neck spitting cobra) showed that *N. nagiricollis* showed higher ACP activity than ALP. But both enzymes needed Mg^{++} to activate them [40]. The enzymes play an important role in liberating the purines, mainly Adenosine, thereby aiding immobilization of the prey organism. The Purines act as multi-toxins inducing hypotension and paralysis [41] *via* purine receptors in the prey's body [42].

5.1.4 Hyaluronidase (Hyl)

Hyaluronidases are a group of enzymes that are responsible for the degradation of Hyaluronic acid (HA), a glycosaminoglycan commonly found in abundance in nervous, epithelial, and connective tissues in all animals. Isolation and biological characterization of Hyl has been done from the venom of many snakes including *N. naja* – Indian Cobra [43], *Agkistrodon contortrix contortrix* – Eastern Copperhead [44], *C. cerastes* – Saharan Horned Viper [45], *Crotalus durissus terrificus* – South American Rattlesnake [46], *Bothrops pauloensis*- South American Pit viper and *Bungarus caerulecs* - Indian Krait. The snake Hyaluronidase (SHyl) from the venom of *Bungarus caerulecs* (Indian Krait) was found to have a molecular weight of 14 ± 2 kDa. The enzyme has an optimum temperature of 37°C and an optimum pH of 6 [47].

The cDNA of SHyl isolated *B. pauloensis* venom gland shows a protein with 194 amino acids synthesized from 1175 bps. The cDNA variants of SHyl isolated *Echis pyramidum leakeyi* (Kenyan Carpet Viper), *Echis carinatus sochureki* (Sochurek's saw-scaled viper) and *Bitis arietans* (Puff Adder) all show the presence of a truncated protein: Hy-L- 1000 that encodes the consensus amino- and carboxyl-termini with a central deletion of 256 residues, Hy-L-750 that lacks the consensus amino-terminus and Hy-L-500 that lacks the amino-terminus and encodes a shorter carboxy-terminal segment [48]. The SHyl is referred to as a 'Spreading factor' as it destroys the extracellular matrix (ECM). By degrading Hyaluronic acid, the enzyme increases the permeability of the tissue paving the way for the other venom toxins to act [49]. Many Hyaluronidase-type proteins have been identified in snake venom. These variants are produced by alternative splicing pathways. The Hyaluronidase-type proteins have not been isolated or characterized as they are highly temperature and pH-sensitive.

5.1.5 Phospholipases

These are enzymes that generally hydrolyze phospholipids into fatty acids and lipophilic substances. There are four major classes named A, B, C and D which are differentiated by the type of reaction they catalyze. Phospholipase A2 (PLA2) is found to be present in the venom of snakes and bees [50]. The enzyme acts on intact lectin molecules and hydrolyses the fatty acids esterified to the second carbon atom [51]. The venom enzymes are like mammalian enzymes in structure and function. The Phospholipase A2 enzymes found in venom are further grouped as I, II and IIE. Group I are major components of Elapidae venom, Group II are major components Viperidae venom [52] and IIE have been identified in the venom of non-front fanged snakes [53]. This enzyme which has a high affinity to specific receptors and

a separate pharmacological site can target a large spectrum of tissues and thereby induce pharmacological effects which are dependent or independent of the catalytic activity of the enzyme.

There exist many unique examples of modulation of PLA2 activity generated by molecular evolution. The enzyme can exist as a homodimer, a post synaptic complex called Vipoxin (South-Eastern European Viper, *Vipera ammodytes meridionalis*). It is composed of PLA2 along with an acidic/catalytic inactive PLA2 like component called the inhibitor (Inh). Both components have 62% sequence homology. It is thought that the Inh acts to stabilize the enzyme component. It could have evolved from the catalytic molecule to the inhibitor [54]. Further studies have shown that a single change in amino acid sequence alters the function of the molecule. Gln48 PL A2 (*V. ammodytes meridionalis*) acts as a chaperone molecule and directs a toxic His48 PLA2 onto an acceptor. Homodimer of Gln48 PLA2 or His48 PLA2 is less toxic when compared to the heterodimer containing both Gln48 PLA2 and His48 PLA2. In another example, neonates of the Mexican jumping viper, *Metapilcoatlus* sp., have been reported to lack PLA2s but in contrast the adults have large quantities of the enzyme. But the venom of both the neonates and the adults was found to be haemorrhagic [55].

5.1.6 L-amino acid oxidases

L-amino acid oxidases (LAAOs) are multifunctional enzymes. They produce hydrogen peroxide and ammonia as part of their catalytic activity. These are highly toxic and can destroy major components of the cell viz. nucleic acids, proteins and the plasma membrane [56]. Snake venom L-amino acid oxidases (SVLAAOs) were first detected in the venom of *Vipera aspis*. SVLAAOs are homodimers with cofactors FAD (Flavin Adenine Dinucleotide) or FMD (Flavin Mononucleotide) linked covalently. Abundance of Riboflavin, also a pigment, is a major contributor to the yellow color of snake venom [57].

SVLAAOs vary between snake species. The enzymes when injected into the prey cause the formation of oxygen reactive species extracellularly. These highly toxic oxygen reactive species, hydrogen peroxide and ammonia, alter the permeability of the plasma membrane and induce apoptosis, which in turn leads to cell death [58]. The SVLAAOs are dependent on ions for activation and inactivation. The LAAOs found in the venom of *Crotalus adamanteus*, Eastern Diamondback rattlesnake, require Mg^{2+} to be activated [59], whereas the enzymes in the venom of *Lachesis muta*, South American Bushmaster, and *Bothrops brazli*, Lancehead pit viper, are inhibited by the binding of Zn^{2+} [60].

Analysis of the sequences of SVLAAOs from around the globe showed ~60% similarity. The most dissimilar regions were the C and N terminals of the protein. Most SVLAAOs are rich in asparagine, glutamic acid and aspartic acid residues. The number of cysteine residues varies implying variation in the tertiary structure of these proteins [61].

5.1.7 Metalloproteinases

Metalloproteinases are typically enzymes that depend on a metal ion to aid their catalytic activity. Snake venom Metalloproteinases (SVMPs) are Zinc (Zn^{2+}) dependent enzymes. Their size ranges from 20 to 110 kDa. They are broadly grouped into three (PI, PII, PIII) based on their structural domains. SVMPs in their varied isoforms are responsible for heaemorrhagic and coagulopathic nature of snake venoms. The SVMPs act on the different stages of the blood clotting pathway [62–63].

5.2 Toxins in snake venom

The myriad of toxins found in snake venom are biologically costly to produce but potent and snakes have invested years of evolution to refine them. Many other toxins are species-specific and have been grouped by their pharmacological action to enable easy study. Though many toxins have been named, the neurotoxins and hemotoxins dominate them all. The identification, isolation characterization and evolution of snake venom toxins have been an area of prolific research since the 1970s.

5.2.1 Neurotoxins in snake venom: three-finger toxin (3FTx) super family

Many of the toxins predominant in snake venom belong to the three-finger toxin (3FTx) family. The group is named for the specific protein fold of three β strand loops connected to a central core with four disulphide bonds. This is a conserved feature. The proteins in this family are at an average of 60 to 74 amino acid residues in length [64]. These 3FTxs are peculiar to snakes although the superfamily of three-fold proteins is common to all eukaryotes [65]. Studies have shown that the 3FTxs of snakes have evolved from non-toxic three-finger proteins [3].

The number of 3FTxs varies from species to species. Elapsid and Colubrid venom are found to be abundant in 3FTxs [66]. 95% of the proteins in the venom of *Micrurus tschudii*, the desert coral snake [67], 70% of the proteins in the venom of *Ophiophagus hannah*, the King Cobra [68] and *Dendroaspis angusticeps*, the Eastern green mamba [69] are 3FTxs. These toxins bind post-synaptically and induce flaccid paralysis in the prey animal.

The structural differences between members of the family are broadly based on the length and number of disulphide bridges. - the longer 3FTxs with a chain length of 66–74 residues with 5 disulphide bridges (Examples: α-neurotoxins, γ-neurotoxins, hannalgesin, κ-neurotoxins) and the shorter chains with a chain length of 57–62 residues with 4 disulphide bridges (Examples: α-neurotoxins, β-cardiotoxins, cytotoxins, fasciculins and mambalgins). The 3FTxs can exist as covalent/non-covalent homo or heterodimers.

The mechanism of action of 3FTxs is varied despite them all having the same 3-finger fold. α-neurotoxins have been shown to inhibit acetylcholine receptors in muscle synapses [70]. κ-neurotoxins on the other hand inhibit acetylcholine receptors in neural synapses [71], fasciculins inhibit acetylcholinesterase [72], mambin interacts with platelet receptors [73], mambaligins inhibit ASIC channels [74] and calliotoxin activates voltage-gated sodium channel [75] to name just a few. It is to be noted that no 3FTxs are involved in inflammation and hyperalgesia typical of other snake toxins. The 3Ftxs target many ion channels and receptors in the prey animal. This is attributed to the unique capacity of the 3-finger fold and its ability to modulate diverse biological functions. Specific amino acid sequences in critical segments of 3FTxs have been identified, these sequences play an important role in binding to the target sites. The interactions of Acetylcholinesterase in the prey with the 3 loops in the fasciculin molecule show the first look of the fasciculin interaction with the outer enzyme but the second loop is inserted in the active site with hydrogen bonding (Lys 25, Arg24, Asn47, Pro31, Leu35 and Ala12) and hydrophobic interactions (Lys32, Cys59, Val34, Leu48, Ser26, Gly36, Thr15 and Asn20) [76]. The interactions of Muscarinic toxins from mamba venom [77], Neurotoxin II (NTII) from the venom of *Naja oxiana*, the Central Asian Cobra [78], Neurotoxin b (NTb) from the venom of O. hannah, King Cobra [79] have all been studied in detail and reports show the importance of the amino acid sequence in binding and modifying the action of the receptors. Any change in these sequences leads to loss of neurotoxicity of the molecule.

5.2.2 Cardiotoxins/cytotoxins

These toxins attack the cardiac muscle preventing muscle contraction. This leads to the irregularity of heartbeat and ultimately stopping of the heart. Experiments have shown that the toxins tend to bind to the surface of the muscle and cause depolarization. These toxins are ample in mamba venom and few species of cobra venom. Other cardiotoxins interact non-specifically with phospholipids [80] or induce insulin secretion [81]. B – cardiotoxins inhibit β-adrenoreceptors [82].

Cardiotoxins are single chain, small molecular weight (~ 6.5 kDa) proteins that are highly basic (pI>10). They exhibit a broad spectrum of pharmacological action. The cardiotoxins share significant sequence homology to neurotoxins yet despite this homology they display remarkably different properties. As many as 52 cardiotoxins have been reported and they have a 90% homology of sequence among themselves [83]. Cardiotoxin III (CTx III, Cytotoxin 3) is a 60-residue long toxin peculiar to the Taiwan Cobra (*Naja atra*). It can induce apoptosis in cells via the release of cytochrome [84]. The structure of cardiotoxin VII4 isolated from *Naja mossambica mossambica*, the Mozambique spitting cobra, was crystalized proving it was a dimer and have a molecular mass of 6715 Da. Studies have shown the cardiotoxin blocked nicotinic acetylcholine receptors [85].

A set of proteins called Cardiotoxin-like basic proteins (CLBP) are found to have homology with cardiotoxins but where cardiotoxins have the triple peptide signature (-I-D-V-) between 39 and 41, CLBPs lack this. Other differences include CLBPs having a Gln at 17 which is absent in cardiotoxins. CLBPs also lack the Met residue needed for activity [86]. These molecules are now being assessed for therapeutic ability.

6. Effects of snake bite and snake venom

Snake bite is a neglected public health issue in many tropical and subtropical countries. According to WHO (2021), an annual record of about 5.4 million snake bites and 1.8 to 2.7 million cases of envenomings has been reported. They have also reported that about 140,000 deaths occur. Bites by venomous snakes can cause acute medical emergencies involving severe paralysis that may prevent breathing, cause bleeding disorders that can lead to fatal hemorrhage, cause irreversible kidney failure and severe local tissue destruction that can cause permanent disability and limb amputation. The more severe effects experienced by the children is because of their smaller body mass [87].

The response of neurotoxicity to snake antivenom is dependent on the type of neurotoxins that the snake possess. Cobra venom contains post-synaptic nerutoxins that produce curare-like effect and hence can be reversed by snake antivenom after clinical effects have developed. While krait venom which contains many pre-synaptic neurotoxins, causes paralysis that is irreversible once developed and hence their response to antivenom is very poor [88, 89].

7. Antivenom

One of the major public health issues in the rural tropics is snake bites. Currently, the only specific treatment available to ameliorate the effect of snake bite is antivenom [90]. Snake antivenom was produced by raising hyperimmune serum

in animals, such as horses. The hyerimmune serum was further purified to produce whole immunoglobulin G (IgG) antivenoms and then fractionated to F(ab) and $F(ab')_2$ antivenoms to reduce adverse reactions and increase efficacy.

A significant challenge in manufacturing of antivenoms is the preparation of the correct immunogens (snake venoms). At present very few countries have capacity to produce snake venoms of adequate quality for antivenom manufacture, and many manufacturers rely on common commercial sources [87]. Poor data on the number and type of snake bites have led to difficulty in estimating needs, and deficient distribution policies have further contributed to manufacturers reducing or stopping production or increasing the prices of antivenoms. Weak regulation and the marketing of inappropriate or poor quality antivenoms has also resulted in a loss of confidence in some of the available antivenoms by clinicians, health managers, and patients, which has further eroded demand.

8. Applications of snake venoms

8.1 Therapeutic implications

Snake venom consists of pharmacologically active proteins and peptides. The snake venoms show a distinct complexity from other animal venoms in that they possess a diverse array of proteins and peptides with wide range of pharmacological and toxicological effects.

8.1.1 Snake venom-based drugs

Based on the pharmacological effects produced, snake venom has been classified into haemotoxic, neurotoxic and cytotoxic venom. Although snake venoms are considered as mini drug libraries, only about 0.01% venom has been characterized. Snake venom is considered a valuable source of new principal compounds in drug discovery. Components of snake venom such as PLA2, serine proteases, metalloproteinase, lectins, l-amino acid oxidases, bradykinin potentiating factors, natriuretic factors, integrin antagonists possess pharmacological properties and exhibit neurotoxicity, myotoxicity, cytotoxicity, hemotoxicity, antimicrobial activity, which in turn exerts its action and disrupts the central and peripheral nervous systems, the blood coagulation cascade, the cardiovascular and neuromuscular systems and the general homeostasis state [5].

Importance of snake venom in medicine dates to thousands of years in Ayurveda, homeopathy and traditional or folk medicines. Cobra venom is used in the ayurvedic treatment of joint pain, inflammation, and arthritis [91] and other body fluids such as blood and bile duct in Chinese medicine [92] and lots of the snake venom-based drugs are available in the market and in clinical trials [93].

Various drugs based on snake venom in the market are Captopril® (Enalapril), Integrilin® (Eptifibatide) and Aggrastat® (Tirofiban) and many more are in the pipeline at pre-clinical or clinical trial stage [94]. Captopril®, approved by FDA in 1981, was the first successful drug derived from snake venom [95]. This drug is a biomimetic of bradykinin-potentiating peptide, isolated from the venom of Brazilian arrowhead viper *Bothrops jararaca*, was discovered by the Nobel prize winner Sir John Vane and its commercial production was taken care of by the pharmaceutical giant Squibb. It finds its use in treating hypertension and cardiovascular disease, where it acts by inhibiting the angiotensin converting enzyme that converts angiotensin I to angiotensin II [96].

Two drugs based on snake venom disintegrins, Aggrastat® (Tirofiban) marketed by Medicure Pharma in the US and Correvio International outside US, and Integrilin® (Eptifibatide) developed by Millennium Pharmaceuticals and co-promoted by Schering-Plow (which are both now part of Merck and Takeda Pharmaceuticals) are used as antiplatelet agents [97]. Aggrastat, belonging to the platelet glycoprotein (GP) IIb/IIIa inhibitors and developed based on the RGD sequence (Arg-Gly-Asp) motif from snake venom disintegrins isolated from the venom of *E. carinatus* is administered to treat heart attack patients [98]. Integrilin, which is used for treating acute coronary syndrome, is a peptide drug which mimics a small portion of the glycoprotein (GP) IIb/IIIa inhibitor barbourin found in the venom of the Southeastern pygmy rattlesnake (*Sistrurus miliarus barbouri*) based on the KGD sequence (Lys-Gly-Asp) [99]. Both Aggrastat® and Integrilin® was approved for medical use by FDA in 1998.

Defibrase®/Reptilase® (Batroxobin), a drug based on the thrombin-like serine protease enzyme isolated from the snake venom of two subspecies *Bothrops atrox* and *Bothrops moojeni* [100] is an approved drug mainly used in China to treat a range of disorders, including stroke, pulmonary embolism, deep vein thrombosis, myocardial infarction and perioperative bleeding. Another drug derived from the venom of *B. atrox*, Hemocoagulase® has been widely used in plastic surgery, abdominal surgery, and human vitrectomy [101]. Exanta® (Ximelagatran) derived from cobra venom, a thrombin inhibitor anticoagulant, is used as blood thinner and thrombin inhibitor [102].

Botrocetin® is a drug that is developed based on the platelet aggregating protein from the venom of *B. jararaca* and it is found to enhance the affinity of the von Willebrand factor A1 domain for the platelet receptor glycoprotein Ibalpha (GPIbalpha) [103]. The thrombin like serine proteinase RVV-V from *Vipera russelli* venom, an activator of factor V of the blood coagulation cascade, is tried for destabilizing and selectively inactivating factor V in plasma [104]. Ecarin, a metalloprotease isolated from the venom of the saw-scaled viper (*E. carinatus*) is used as prothrombin activator [105].

8.1.2 Putative therapeutic substances

Taipoxin, a powerful presynaptic neurotoxin from *Oxyuranus scutellatus* (Australian taipan) snake venom, consists of three polypeptides, referred as alpha, beta, and gamma subunits. Trypsin degradation of the β-subunit yields Oxynor which has pharmacological properties against wounds [106]. Oxynor was subjected to clinical development by Ophidia Products, Inc., but no further progress has reported in literature.

Vicrostatin (VCN) is a chimeric disintegrin, made by the fusion of echistatin and contortrostatin, seen in crotalids snake venom. When VCN, packaged in liposome (LVCN), was intravenously administered *in vivo* to breast cancer models, a delayed tumor growth and prolong animal survival was observed [107]. The drug was in pre-clinical studies by Applied Integrin Sciences Inc., but no further progress has reported in literature.

In vitro studies of Salmosin, a disintegrin of 7.8 kDa (73 residues), isolated from *Agkistrodon halys brevicaudus* (Korean mamushi) venom, demonstrated its capacity to inhibit the proliferation of bovine capillary endothelial cells, induced by bFGF (basic fibroblast growth factor) by competing with ECM for binding with αvβ3, detaches cells, and inactivates FAK-dependent signaling pathways, thereby leading to apoptosis [108]. Hence, Salmosin could be used as an anticancer agent in future.

Hannalgesin, an α-neurotoxin of approximately 7.9 kDa (72 residues) isolated from *O. hannah* (King cobra) venom, exhibits analgestic effect through nitric oxide or opioid systems. Its analgesic effect is higher than morphine [109].

8.2 Diagnostics

The feature of not being not affected by therapeutic or physiological coagulation inhibitors [Marsh, 2002], it has been applied for the analysis of hemostatic parameters, such as fibrinogen (dysfibrinogenemia, its breakdown products), antithrombin III, prothrombin (dysprothrombinaemias), von Willebrand factor (vWF), blood clot- ting factors (V, VII, X), protein C (PC), activated protein C (APC), and lupus anticoagulants (LA) [110]. Protac® and Proc Global assay, reptilase® and reptilase time, Anti-nAChR antibodies assay, textarin time, botrocetin®, RVV-V, RVV-X, and dRVVT (dilute Russell's viper venom time), *etc.* are the tests available [111].

8.3 Biochemical tool

The structures, functions and molecular mechanisms of receptors/ ion-channels that exhibit high potency, selectivity, and efficacy can be studied using snake venom peptides as molecular probes [112]. α- neurotoxins such as erabutoxin, α-cobratoxin, and α- bungarotoxin have high affinity for nicotinic acetylcholine receptors (nAChR). This feature is applied in isolating the α-bungarotoxin, from *Bungarus* sp. [113]. The muscarinic neurotoxins (MTs) or mamba toxin produced by *D. angusticeps* (green mamba) and related species is composed of 64–66 amino acids, homologous to α-neurotoxins and are highly selective for muscarinic receptor subtypes (mAChRs) [114]. This study gains importance in studying the role of mAChRs in Alzheimer's disease using mamba toxin and used as therapeutic agent in treating Alzheimer's and Parkinson's disease as it selectively blocks the receptor sub-types [115]. Dendrotoxins and related proteins, from *Dendroaspis* species (mamba snakes), belonging to sub-family of voltage-dependent potassium channels, are homologous to Kunitz-type serine protease inhibitors, composed of 57–60 amino acids polypeptide chain that is stabilized by the presence of three disulphide bridges. Therefore, these toxins could serve as biochemical tools to study various sub-type of L-type calcium channels.

9. Conclusions

Snake venoms are complex mixtures of toxins that exhibit interspecies and intraspecies variation due to the rapidly evolving and diverging venom genes in relation to the geographical area, environmental niches etc. The efficacy of snake venom is influenced by these variations. Future implications on the venom study are in the direction for search of effective pharmacological and diagnostic products.

Conflict of interest

"The authors declare no conflict of interest."

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