African Journal of
Biochemistry and Molecular Biology Research

ISSN : 3434-6120

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJBMBR.v2i1.4931

Snake Venom: Its Biochemical Components and Their Uses in Medicine

Isaac John Umaru & Wisdom L. Benjamin
Federal University Wukari, Taraba State, Nigeria
umaruisaac@gmail.com

Article Info:

Submitted:	Revised:	Accepted:	Published:
Jan 5, 2025	Jan 20, 2025	Feb 2, 2025	Feb 7, 2025

Abstract

Snake venoms have components with diverse biological actions that are extensively studied to identify elements that may be useful in biomedical sciences. In the field of autoimmunity and rheumatology, various findings useful for the study of diseases and potential drug development have been reported. The study of disintegrins, proteins that block the action of integrins, has been useful for the development of antiplatelet agents and principles for the development of immunosuppressants and antineoplastic. Several proteins in snake venoms act on the coagulation cascade, activating factors that have allowed the development of tests for the study of coagulation, including Russell's viper venom time, which is useful in the diagnosis of antiphospholipid syndrome. Neurotoxins with either pre- or postsynaptic effects have been used to study neurogenic synapses and neuromuscular plaques and the development of analgesics, muscle relaxants and drugs for neurodegenerative diseases. Various components act by inhibiting cells and proteins of the immune system, which will allow the development of anti-inflammatory and immunosuppressive drugs. This review summarizes the usefulness of the components of snake venoms in the fields of autoimmunity and rheumatology, which can serve as a basis for diverse translational research

Keywords: Snake, Venom, Biochemical, Components, Medicine

Introduction

Snake venom, which contains zootoxins and is a highly modified form of saliva, aids in the capture and digesting of prey as well as defence against threats. After a bite, it is injected into the target via specialized fangs. The ability to spit or spew poison is possessed by a variety of snakes (Bauchot, 1994). These zootoxins are produced by modified parotid salivary glands, which are typically situated on each side of the head, beneath and behind the eyes, and enclosed in a muscular sheath. Large alveoli are present in the glands, where the generated venom is first kept before being transported through duct to the base of tubular fangs, where it is then expelled (Bottrall, *et al*., 2010).

The only medical therapeutic agent for treating a snakebite is antivenom, however it frequently causes hypersensitivity reactions and offers little protection against the venom's ability to cause hemorrhage, necrosis, and nephrotoxicity (Mattison, 2007). It takes time, money, and proper storage conditions to generate antivenom in animals. Health centers are typically spread out and sparsely populated, and monovalent antivenom and antisera are typically unavailable. There is a pressing need to research and develop new, effective antidotes for snakebite in order to address these shortcomings. According to estimates from the World Health Organization, traditional medicine serves the primary healthcare needs of 80% of the global population. Plants and plant products have been utilized in traditional medicine around the world for as long as man has been aware of them to treat a variety of ailments. According to estimates by Hodgson *et al.* (2002), roughly 70% of the world's population still uses medicinal plants as their main source of medication. Recent advancements in the discovery of analgesic, anti-arthritis, antibacterial, and anticancer agents show the continued significance of plant species in the pharmaceutical industry. The pharmacological properties of numerous plants and plant components are currently being studied, particularly those that are utilized in traditional and folk medicine to treat various ailments. Despite the existence of antiserum, numerous attempts have been undertaken over the years to generate snake venom antagonists, particularly from botanical sources.

Scientists throughout the world have been enthralled by the biochemical makeup and evolutionary histories of animal venoms for decades. Over millions of years, animals that produce venom have improved, developed, and evolved their venom to help them catch prey or defend themselves from predators. As one of the best-characterized animal venoms, snake venom is thought to have first evolved during the Cenozoic Era (Fry, 2005; Fry *et al.*, 2006). It is made up of a complex mixture of pharmacologically and lethally active proteins and peptides (Casewell *et al.*, 2013; Chan *et al.*, 2016). Snake envenomation is a major health and financial liability on a global scale. According to estimates from Kasturiratne *et al.* (2008) and Gutierrez *et al.* (2017), there are 1.8–2.7 million snake bites worldwide each year, resulting in 81,410–137,880 deaths. The World Health Organization (WHO) officially identified snakebites as a priority neglected tropical disease in 2017 (WHO, 2018). Individuals under 30 years old, who are frequently the most economically productive members of a community, suffer the most morbidity and mortality from snakebites.

When compared to the venoms of other species like spiders, scorpions, and cone snails, snake venoms are noticeably more complicated (Zelanis and Tashima, 2014). Disulfide bridged peptides are principally responsible for the pharmacological effects of these animal venoms, but snake venoms contain a more varied assortment of bigger proteins and peptides, leading to a wider diversity of pharmacological and toxicological effects (Zhang, 2015). According to several studies (Vonk, *et al.*, 2011, Slagboom, *et al.*, 2017, Tasoulis and Isbister, 2017), these venoms contain 50–200 components that are spread among dominant and secondary families and can take the shape of different proteins and peptides. The secondary families include cysteine-rich secretory proteins, Lamino acid oxidases, kunitz peptides, C-type lectins, disintegrins, and natriuretic peptides. The dominant families are secreted phospholipases A2 (PLA2s), snake venom metalloproteinases (SVMP), snake venom serine proteases (SVSP), and three-finger peptides (3FTX) (Slagboom, *et al.*, 2017; Tasoulis and Isbister, 2017; Munawar, *et al.*, 2018).

The primary toxins that cause these effects are the PLA2s, SVMPs, SVSPs, and 3FTXs. These poisons, either alone or in combination, are to blame for the various pharmacological effects observed in snake bite victims. Some PLA2s and 3FTX can act on pre- or post-synaptic junctions as antagonists of ion channels and nicotinic or muscarinic receptors to cause severe neurotoxicity, including paralysis and respiratory failure, according to several studies (Fry, *et al.*, 2003; Lynch, 2007; Casewell *et al.*, 2013; Harris and

Scott-Davey, 2013; Tsetlin, 2015). Other PLA2s and 3FTXs also harm local tissue, resulting in swelling, blistering, bruising, and necrosis, as well as systemic consequences such hypovolemic shock (Gutierrez and Rucavado, 2000; Gutierrez, *et al.*, 2005; Harris and Scott-Davey, 2013; Rivel *et al.*, 2016). These substances also harm SVMPs. Additionally, SVSPs and SVMPs cause coagulopathy, hypotension, and bleeding, among other hemostatic and cardiovascular consequences (Slagboom, *et al.*, 2017). It's interesting to note that some PLA2s, SVSPs, and SVMPs can also cause severe pain by modifying pain pathways by activating ion channels like transient receptor potential vanilloid type 1 (TRPV1) and acid-sensing ion channel (ASIC) (Bohlen, *et al.*, 2011; Zhang, *et al.*, 2017) and/or by pain sensitization through inflammatory mediators (Zychar, *et al.*, 2010; Menaldo, *et al.*, 2013; Ferraz, *et al.*, 2015; Mamede, *et al.*, 2016; Zambelli, *et al.*, 2017b). The inflammation induced by the elapid and viper venoms is usually reported to cause pain or hyperalgesia in human and in experimental models (Hifumi, *et al.*, 2015; Bucaretchi, *et al.*, 2016; Kleggetveit, *et al.*, 2016; Mamede, *et al.*, 2016). Unfortunately, these are not completely reversed by antivenom and anti-inflammatory therapies (Picolo, *et al.*, 2002; Ferraz, *et al.*, 2015; Hifumi, *et al.*, 2015).

Currently, intravenous antivenom injection in conjunction with analgesics, hydration therapy, hemodialysis, and/or antibiotics is used to treat the toxicological consequences caused by snake envenomation (Gutierrez, *et al.*, 2017). The persistently high rates of clinical disease and mortality linked to snake envenomation globally have made snakebite treatments challenging, while being adequate in the majority of patients (WHO, 2018). Additionally, the long-term effects of snake envenomation have been underreported. Many victims have complained of persistent symptoms in the area where they were bitten, including complex regional pain syndrome (CRPA) and musculoskeletal impairments (Jayawardana *et al.*, 2016). Limited para-specificity, poor antibody specificity, a high incidence of adverse reactions, a lack of availability and affordability for those who need them, and poor efficacy against local tissue effects are all issues with currently available snakebite treatments (Williams *et al.*, 2011; Gutierrez *et al.*, 2017; Ainsworth *et al.*, 2018; Harrison *et al.*, 2019). In order to better combat the severe acute and long-term effects of snake envenomation, current research efforts should focus on the development of more effective snakebite therapeutics capable of generically totally inhibiting the key toxic components of snake venoms.

Therefore, in light of the public health importance and the complexity of snake venoms, the aim of this seminar paper is to review the biochemical components of snake venom and their uses in medicine viz a viz uses and side effects of antivenom

Snake Venom

According to Soares, *et al.*, (2004), snake venom is the secretion of venomous snakes that is produced and stored in venom glands. The zootoxin-secreting glands, which are modified parotid salivary glands, are located on each side of the head beneath and behind the eyes and are enclosed in a muscular sheath. The venom is first stored in the glands' enormous alveoli before being transported by the duct to the tubular fangs, where it is then injected. When consumed, the many proteins, peptides, and enzymes that make up snake venom are usually not harmful. Phospholipase A2 (PLA2s), myotoxins, hemorrhagic metalloproteinases and other proteolytic enzymes, coagulant components, cardiotoxins, cytotoxins, and neurotoxins are only a few of the harmful proteins found in snake venom (Leon, *et al.*, 2011). Snake venoms are mixtures of non-enzymatic proteins and enzymes that are used to immobilize and digest prey. These enzymes provide biochemists and enzymologists with fascinating models because of their improved catalytic efficiency, thermal stability, and resistance to proteolysis. (Ahmed. 2009).

Proteins that function as pre-digestive and paralyzing digestive enzymes. Therefore, digestion begins outside the predator's alimentary canal while immobilizing the prey. The snake then swallows its prey entire, liquefying most of the tissue and discarding the feces and any parts that could not be digested (feathers, hair, claws). Although venom is used by venomous snakes to capture prey, they also use it to fan out their bites in defense against fearsome predators and aggressors.

Role of Snake Venom

In terms of biochemistry, snake venoms are complex mixtures of pharmacologically active proteins and polypeptides that all work together to help in capturing the prey. Snakes use their venoms as offensive weapons in incapacitating and immobilizing their prey (the primary function), as defensive tools against their predators (the secondary function), and to aid in digestion. Many protein toxins have been isolated and studied in snake venoms (Bauchot, 1994).

Types of Snake Venom

Remarkably, snake venom composition show a discrepancy interspecifically (Fry, *et al.*, 2008; Tasoulis and Isbister, 2017), as well as intraspecifically, with several factors prompting this diversity including age (Dias, *et al.*, 2013), gender (Menezes, *et al.*, 2006; Zelanis, *et al.*, 2016), location (Durban, *et al.*, 2011; Goncalves-Machado, *et al.*, 2016), diet (Barlow, *et al.*, 2009), and season (Gubensek, *et al.*, 1974). This variability phenomenon reinforces toxin diversity and multifunctionality, and is of great importance to be considered in antivenom production and envenomation treatment (Gutierrez, *et al.*, 2017).

Due to the inability of antivenoms' particular antibodies to neutralize the heterologous toxins present in various venoms, venom variety can have detrimental effects on snake bite patients (Casewell, *et al.*, 2012). distinct species have distinct types of venom, which vary depending on their species, habitat, age, and other factors.

Pharmacologically, however, there are three main categories for snake venoms: hemotoxic, neurotoxic, and cytotoxic (WHO, 2010).

1) Haemotoxic venoms have biochemical components which affects cardiovascular system,
2) Cytotoxic venoms which targets specific cellular sites and
3) Neurotoxic venoms which harms the nervous system of human body.

According to Jin and Varner (2004), enzymes found in snake venom catalyse the breakdown of proteins and membrane components, causing tissue necrosis and blood coagulation. Since the beginning of time, humans have been fascinated with snakes. They are among the only living things that can elicit a favourable or negative reaction when a hissing, rattling, or even just the term "snake" is mentioned (Shenoy *et al.*, 2013). The fatal effect of their venoms, which, when injected into the victim, cause a variety of physiological reactions like paralysis, myonecrosis, and frequently death, is likely what causes this great interest. To aid in prey capture, snake venoms have developed into complex combinations of pharmacologically active proteins and peptides that have strong, deadly, and incapacitating effects. According to Debnath *et al.* (2007), their diet is quite diverse and includes tiny animals including snails, fish, frogs, toads, lizards, chickens, mice, rats, and even other snakes.

Table 1. Some Snakes and Their Type of Venom

Common krait 	The venom of this snake is neurotoxic.
Russell's viper 	Its venom is haemotoxic
Spectacled Cobra 	Its venom is neurotoxic

Biochemistry and Physiology of Snake Venom

In the 1800s, it was discovered that venom is a protein-rich mixture, with proteins making up around 90 to 95% of its dry weight. The biological effects of the venom are also caused by these proteins. These proteins can be classified as toxins and enzymes (Ferraz, *et al.,* 2019). Within a species, the components of venom might vary from animal to animal. Age (Dias, *et al*., 2013), gender (Zelanis, *et al*., 2016), location (Gonçalves-Machado, *et al*.,

2016), prey species/diet (Barlow, *et al*., 2009), and season (Gubensek, *et al*., 1974) have all been demonstrated to have an impact on venom composition. The proteins that make up venom are all recycled from normal physiological processes. The discovered venom proteins have been investigated as single proteins, protein complexes, and protein families. The proteins in the complex might be homodimers (consisting of the same subunits) or heterodimers (consisting of various subunits, sometimes from various families). Covalent bonds hold these complexes together, and the complexes are pharmacologically more effective than the individual proteins or enzymes. Critical residues that may otherwise have been hidden in the individual enzymes appear to be exposed by the complexes (Doley *et al*., 2009).

Enzymes in Snake Venom

The most prevalent snake venom enzymes include phospholipase A2s (PLA2s), serine proteinases, metalloproteinases, acetylcholinesterases (AChEs), l-amino acid oxidases, nucleotidases (5' nucleotidases, ATPases, phosphodiesterases, and DNases), and hyaluronidases. Numerous substances included in snake venom often work together to immobilize animals and start the digestive process. There are numerous enzymes, and some toxins even function as enzymes. The role of the most extensively researched enzymes is mentioned here.

5' Nucleotidase (5'-NT)

This enzyme, which has a molecular weight of 61 kDa and 548 amino acids, is present in practically every living cell. Nucleosides are hydrolyzed by the enzyme. All snake venom around the world contains it. There have also been isolated isoforms, such as the homodimeric monomer with a molecular mass of 60 kDa that was discovered in the venom of the Cypriot blunt-nosed viper (*Vipera lebetina*). According to Trummal, *et al*., (2015), this isomer prevents platelet aggregation caused by ADP or collagen. The enzyme has two Zn + binding sites, and the isomer identified from the Japanese pit viper (*Gloydius blomhoffii blomhoffii*) demonstrates that it can exist as a trimer or a tetramer. (Ogawa, *et al*., 2008). Studying the venom of three snakes in Pakistan that are members of the Viperidae family—the *Vipera russelli*-Russell's viper, *Echis carinatus*-the Indian saw-scaled viper, and the *Eristocophis macmahonii*-the Asian sand viper—found that all three had significant levels of 5'-NT activity (Mahmood, *et al*., 1996). The study snakes in Brazil with the highest 5'-NT activity were *Bothrops brazili* (Brazil's lancehead). On the other hand, the 5'-NT activity in

the venom of the South American green racer snake, *Philodryas olferssi*, was negligible to non-existent. It has been proven that the 5'-NT enzyme can work in concert with other enzymes to have a strong anti-coagulant impact. Adenosine is reported to be released, which aids in the prey's immobilization. It was demonstrated in 2008 that either the entire enzyme or a portion of it is secreted in the venom. The ectodomain of the enzyme is cleaved in the venom gland or other specialized tissues to release the soluble form (Ogawa, *et al*., 2008).

Acetylcholinesterase (AChE)

It is the main enzyme in snake venom that, among other related neurotransmitters, catalyzes the body's breakdown of acetylcholine into acetate and choline. One of the most thoroughly researched enzymes from snake venom is called AChE, and detailed structural information has been provided. All snakes, with the exception of mambas, have been found to contain high amounts of this enzyme, notably Elapid snakes (Cousin X, Bon C., 1999). Although the enzyme is present, no activity was found in the venom of Viperidae or Crotalidae snakes. With an average of 8 mg/g of dried weight, *Bungarussp* has the highest venom AChE (VAChE) concentration (Frobert *et al*., 1997). At pH 8.5 and 45° C, VAChE is most active, according to research. (Ahmed, *et al.,* 2012).

With a few significant differences, the protein structure of VAChE exhibits homology to Torpedo and mammalian AChE. These modifications make VAChE less complex structurally than membrane-bound AChE. There are numerous isoenzymes that can be distinguished solely based on charge (Lee *et al*., 1977). The protein structure of *Bungarus fasciatus* (BfAChE) and *Naja oxiana* (NnAChE) VAChE has been investigated. BfAChE is a hydrophobic monomer that is soluble in water. An alternate exon ('S') is present in the C terminal peptide. The final 8 of its 15 residues are removed to create the mature protein. BfAChE differs from mammalian AChE in the following ways: Acidic residue 285 (glutamate/aspartate) is replaced by Lys285, while Tyr70 is replaced by Met70. According to Rosenberry. *et al.* (2005), the active site gorge is 20 A° deep and has two ligand-binding sites. The co-existence of an open/closed condition in the rear door channel and partially obscured gorge entrance is demonstrated by the crystalline structure. The existence of Met70 widens the gorge's entrance, improving binding. Despite having a non-amphiphilic C terminus, the subunit can nevertheless form conventional dimers.

At 0.2 mg/ml as a monomer and 2 mg/ml as a dimer, the NnAChE is present. It has a molecular weight of 67,000–2000 Da, a single polypeptide chain, and can be found in many isoforms with various isoelectric points (Raba *et al*., 1979). According to Bourne *et al.* (2015), it varies from BfAChE by possessing a dimerization domain where His takes the role of Pro at position 514. Acute neuromuscular paralysis and neuromuscular weakening have been linked to VAChE. This can be the result of faulty communication at the neuromuscular junction. (Ferraz *et al* 2019). Acetylcholine produced from synaptic vesicles may be immediately hydrolyzed with the help of AChE in elapid venoms. This release may have been caused by neurotoxic to evade postjunctional receptors' competing protection from acetylcholine against neurotoxin (ZELLER, 1950).

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

These enzymes are present in lysosomes and during digestion they catalyse the removal of phosphoryl groups from molecules. They are present in all snake venoms. Both enzymes have a greater activity in Elapidae than Viperidae snakes. In a study (Hassan, *et al.,* 1981), that compared *Cerastes cerastes* (Saharan horned viper), *Cerestes vipers* (Saharan sand viper), *Naja haje* (Egyptian cobra) and *N. nigricollis* (Blacked neck spitting cobra), *N. nagiricollis* showed higher ACP activity than ALP. But both ACP and ALP enzymes needed Mg++ to activate them. These enzymes are essential in liberating the purines (mainly Adenosine), thereby aiding immobilization of the prey. As a result of liberation, the purines act as multi-toxins inducing hypotension and paralysis (Aird, 2002) via purine receptors in the prey's body (Sawynok, 2007).

Hyaluronidase (Hyl*)*

A group of enzymes known as hyaluronidases are in charge of breaking down hyaluronic acid (HA), a glycosaminoglycan that is frequently present in high concentrations in the connective, epithelial, and neurological tissues of all mammals. Many snakes, such as the *N. Naja* - Indian Cobra (Girish, *et al*., 2004), the *Agkistrodon contortrix contortrix* - Eastern Copperhead (Wagstaff, *et al*., 2009), *C. cerastes* – Saharan Horned Viper (Wahby *et al.,* 2012), *Crotalus durissus terrificus* – South American Rattlesnake (Bordon *et al.,* 2-012), *Bothrops pauloensis*- South American Pit viper and *Bungarus caerulecs* - Indian Krait and others have had their venom isolated and biologically characterized. The molecular weight of the snake hyaluronidase (SHyl) from the venom of Bungarus caerulecs (Indian Krait) was discovered

to be 14 2 kDa. The enzyme functions best at a temperature of 37 °C and a pH of 6 (Bhavya, *et al.,* 2016).

The cDNA of the SHyl extracted venom gland of *B. pauloensis* reveals a protein with 194 amino acids, which were produced from 1175 bps. The cDNA variants of the SHyl isolated *Echis pyramidum leakeyi* (Kenyan Carpet Viper), *Echis carinatus sochureki* (Sochurek's saw-scaled viper), and *Bitis arietans* (Puff Adder) all demonstrate the presence of a truncated protein: Hy-L-1000, which encodes the consensus amino- and carboxyltermini 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 (Harrison, *et al.,* 2009). The extracellular matrix (ECM) is destroyed by the SHyl, which is why it's called to as a "Spreading factor." The enzyme improves the tissue's permeability by breaking down hyaluronic acid, allowing the other venom poisons to take effect (Eulalio, *et al*., 2014). In snake venom, numerous proteins of the hyaluronidase class have been discovered. Alternative splicing pathways result in the generation of these variations. Due to their severe temperature and pH sensitivity, the proteins of the hyaluronidase-type have not been isolated or described.

Phospholipases

These are the kinds of enzymes that hydrolyze phospholipids into fatty acids and other lipophilic compounds in most cases. The type of reaction that each of the four primary classes—A, B, C, and D—catalyzes distinguishes them from one another. According to Gopalakrishnakone *et al.* (2016), PLA2 was discovered to be present in the venom of bees and snakes. The enzyme hydrolyzes fatty acids esterified to the second carbon atom when it interacts with intact lectin molecules (Kini, 2003). The Phospholipase A2 enzymes found in venom are further grouped as I, II, and IIE, with Group I being major components of Elapidae venom, Group II being major components of Viperidae venom, and Group IIE having been identified in the venom of non-front fanged snakes (Perry, *et al*., 2018). Venom enzymes are similar to mammalian enzymes in structure and function. This enzyme can target a broad range of tissues and produce pharmacological effects that are dependent or independent of the catalytic activity of the enzyme because it has a high affinity for particular receptors and a separate pharmacological site.

There are numerous distinct instances of molecular evolution-driven PLA2 activity regulation. The South-Eastern European Viper, *Vipera ammodytes meridionalis*, which

contains the enzyme as a homodimer is known as Vipoxin, a post-synaptic complex. It is made up of PLA2 and an inhibitor (INH), an inactive acidic/catalytic PLA2-like substance. Both components share 62% of the same sequence. The enzyme component is thought to be stabilized by the INH. It might have transitioned from the inhibitor to the catalytic molecule (Betzel, *et al*., 1999). Additional research has revealed that a single modification to the amino acid sequence can impact how a molecule behaves. A hazardous His48 PLA2 is directed onto an acceptor by the chaperone molecule Gln48 PL A2 (*V. ammodytes meridionalis*). When compared to a heterodimer that contains both Gln48 PLA2 and His48 PLA2, homodimers of Gln48 PLA2 or His48 PLA2 are less hazardous. Another illustration is the Mexican jumping viper, *Metapilcoatlus sp*., whose neonates are believed to be deficient in PLA2, in contrast to the adults, who have abundant amounts of the enzyme. But it was discovered that the venom of both the adults and the neonates was hemorrhagic (García-Osorio, *et al.,* 2020).

According to Harris and Scott-Davey (2013), phospholipases A2 are a key factor in the neurotoxic and myotoxic consequences of snake bites. These proteins have molecular weights of 13–15 kDa. In addition, a third class of PLA2s, known as IIE, have primarily been found in the venom of snakes without front fangs, though it is unknown how significant a role they play in the venom arsenal. Each of these PLA2s kinds (I, II, and IIE) have been individually recruited into snake venom systems, according to studies recreating the evolutionary history of this multi-locus gene family, indicating that their deadly qualities have evolved by convergent evolution. Despite having similar enzymatic capabilities, PLA2s from vipers and elapids have undergone distinct levels of significant gene duplication throughout the course of evolution, which has led to differing patterns of residue conservation. Additionally, group II PLA2 isoforms that are catalytically active (like Asp49) and catalytically inactive (like Lys49) are found in the venom of viper snakes. Many of these toxins also promote inflammation, including edema formation, cytokine production, and leukocyte recruitment, pain by inducing thermal allodynia and mechanical hyperalgesia, paralysis through block of neuromuscular transmission, and intensify hemorrhage by inhibiting coagulation. A number of PLA2s have strong myotoxic effects that frequently result in severe necrosis. Pre-synaptic terminals and sensory nerve endings are modulated by these toxins, which causes neurotoxic effects as well as some proinflammatory consequences. Furthermore, PLA2s from *Crotalus durissus cascavella* and *Naja mossambica* have been shown to elicit substance-P mediated neurogenic inflammation.

It's interesting to note that macrophages can be activated by the C-terminal of Myotoxin-II, a Lys49-PLA2 identified from *Bothrops asper*. This suggests that this region may be essential for the observed enzymatic-independent inflammation. PLA2s cause pain by activating sensory neural processes and inducing inflammatory reactions. A 500-fold reduction in enzymatic activity had only a small impact on the neurotoxicity of the PLA2 OS2 from the Australian Taipan snake *Oxyuranus scutellatus scutellatus*, according to a thorough mutational research. The N- and C-terminal portions of OS2 were also necessary for its enzymatic activity, and the N-terminal region played a significant part in the neurotoxicity of the central nervous system. *Bothrops jararacussu* (BThTx-I) Lys49-PLA2 alanine imaging revealed discrete areas implicated in the hyperalgesia and edema. The presence of basic and hydrophobic residues in this C-terminal region has been substantially linked to PLA2s' capacity to bind with one another and pierce the lipid bilaye. A multitude of factors, such as the evolutionary history, phylogeography, diet, and/or environmental conditions pertaining to a species or populations within a species, may be reflected in the heterogeneity of PLA2 isoforms found in the venom of various snakes. The multi-locus gene families that code for many snake venom poisons have been discovered arranged in arrays on microchromosomes, most likely as a result of tandem gene duplication events. Many snake venom toxin families, including the PLA2s, have evolved through a process of gene duplication and loss. Duplications are thought to have initially stimulated a gene-dosing effect while also releasing duplicates from evolutionary constraints, allowing for a scenario that may facilitate protein sub- and/or neo-functionalization (Lynch and Conery, 2000). Studies have shown that major genomic differences in PLA2 toxin loci, with variation at different gene complexes leading to noticeably different haplotypes, are at least partially responsible for the extremely divergent venom phenotypes (e.g., neurotoxic vs. haemorrhagic) observed within populations of the same snake species or between closely related species. Although natural selection influenced by environmental conditions and hybridization events have both been hypothesized, the precise mechanisms underlying such variety remain unknown.

L-amino acid oxidases

LAAOs, or L-amino acid oxidases, are enzymes with numerous uses. They generate ammonia and hydrogen peroxide as a result of their catalytic activity. Nucleic acids, proteins, and the plasma membrane are among the important cell components that can be destroyed by these since they are so toxic (Findrik, *et al.*, 2006). The venom of the *Vipera*

aspis was the first to have snake venom L-amino acid oxidases (SVLAAOs). SVLAAOs are homodimers with covalently attached flavin mononucleotide (FMD) or flavin adenine dinucleotide (FAD) cofactors. The yellow hue of snake venom is mostly caused by an abundance of riboflavin, a pigment (Fernandes, *et al.*, 2014).

Different snake species have different SVLAAOs. When the enzymes are introduced into the prey, extracellularly formed oxygen reactive species result. Hydrogen peroxide and ammonia, two extremely harmful oxygen reactive species, change the plasma membrane's permeability and cause apoptosis, which results in cell death (Ande, *et al.*, 2008). Ions are required for both activation and inactivation of the SVLAAOs. While the enzymes in the venom of *Lachesis muta,* the South American Bushmaster and *Bothrops brazil,* the Lancehead pit viper are inhibited by the binding of Zn2+ (Solis, *et al.,* 1999), the LAAOs identified in the venom of the Eastern Diamondback rattlesnake, *Crotalus adamanteus*, require Mg2+ to be activated (Paik & Kim.,1965).

Around 60% commonality was found when SVLAAO sequences from different parts of the world were analyzed. The C and N terminals of the protein were the most different areas. Asparagine, glutamic acid, and aspartic acid residues are prevalent in the majority of SVLAAOs. Variations in the tertiary structure of these proteins are suggested by the variance in the number of cysteine residues (Stábeli, *et al.*, 2004).

Metalloproteinases

Metalloproteinases are normally enzymes whose catalytic activity is aided by a metal ion. snake venom Metalloproteinases (SVMPs) are enzymes that require zinc (Zn2+). They are between 20 and 110 kDa in size. On the basis of their structural domains, they are broadly divided into three (PI, PII, and PIII). The hemorrhagic and coagulopathic properties of snake venom are caused by SVMPs in their various isoforms. The many phases of the blood clotting pathway are affected by the SVMPs. (Kini & Koh., 2016; Slagboom *et al.*, 2017).

These toxins are important components of viper venoms and are crucial to the toxicity of the venoms produced by these snakes. Venom SVMPs are descended from disintegrin and metalloproteinase (ADAM) proteins, notably ADAM28, with PIII being the most primitive structural form made up of metalloproteinase, disintegrin-like, and cysteine-rich domains. Later, P-II SVMPs split off from P-IIIs and are made up of a metalloproteinase and a disintegrin domain. The latter is frequently seen in venom as a

byproduct of proteolytic processing. As a result of the loss of the P-II disintegrin-encoding domain, the final class, PI SVMPs, which only include the metalloproteinase domain, seems to have developed on numerous independent occasions in certain lineages. SVMPs exhibit evidence of numerous gene duplication events associated with bursts of fast molecular evolution across this diversified evolutionary history. Only P-III SVMPs, however, have been found in elapid and "colubrid" venoms, despite the fact that all three classes of SVMPs have been characterized from viper venom. These P-III SVMPs can play a significant role in "colubrids," although normally constituting just 10% of the venom toxins in elapid snakes. These abundance differences likely underpin the distinct pathologies observed followi ings caused by snakes in these families. The diversity of SVMP isoforms frequently seen in viperid snake venom likely facilitates synergistic effects, such as simultaneous action on numerous phases of the blood clotting cascade. SVMPs contribute significantly to the hemorrhagic and coagulopathic venom activities after bites by viperid snakes. Even though they lack the sub-class diversity seen in vipers, some "colubrid" snakes, whether they are medically significant or not, exhibit signs of having several SVMP toxins in their venom. However, elapid snakebites rarely result in systemic hemotoxicity, which is probably because these venoms typically have little variety or quantity of SVMPs and are predominated by neurotoxic toxin families such the 3FTXs and PLA2s. As was already indicated, SVMPs are renowned for their hemorrhagic activity as well as their capacity to affect several phases of the blood coagulation cascade, leading to a deadly combination of systemic hemorrhage and incoagulable blood in prey and/or victims. According to research, SVMP-induced hemorrhage is caused by a mechanism that involves two phases. To weaken the capillary arteries, SVMPs first rupture the basement membrane and adhesion proteins of the endothelial cells-matrix complex. In the second stage, the endothelial cells separate from the basement membrane and become incredibly thin, causing the capillary walls to rupture and blood to leak out of the thin, vulnerable capillary walls. SVMPs affect homeostasis in addition to their proteinase activity by changing coagulation, which adds to their lethal hemorrhagic consequences. This happens by altering the coagulation cascade-mediating factors like fibrinogenase and fibrolase, consuming pro-coagulation factors like Factor X, prothrombin, and fibrinogen, inhibiting platelet aggregation, and engaging in inflammatory processes.

Toxins in Snake Venom

The numerous poisons present in snake venom are difficult for living things to manufacture, but they have undergone years of evolution to become potent and effective. Many other toxins are species-specific and have been categorized for easier investigation based on their pharmacological activity. Despite the fact that many toxins have been identified, neurotoxins and hemotoxins are the most common. Since the 1970s, there has been a lot of research into the identification, isolation, characterization, and evolution of snake venom toxins.

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

The three-finger toxin (3FTx) class of toxins includes several of the poisons that predominate in snake venom. The name of the group refers to a particular protein shape that consists of three strand loops that are joined to a central core by four disulfide connections. This feature has been preserved. According to Kessler *et al.* (2017), the length of the proteins in this family ranges from 60 to 74 amino acid residues on average. Although the superfamily of three-fold proteins is shared by all eukaryotes, these 3FTxs are unique to snakes (Reyes-Velasco *et al.*, 2015). According to research (Casewell *et al.*, 2013), snakes' 3FTxs evolved from non-toxic three-finger proteins. The quantity of 3FTxs differs between species. Venom from elapsid and colubrid species is shown to be prevalent in 3FTxs (Barber, *et al.,* 2013). According to Sanz *et al.* (2016), 95% of the proteins in the venom of the desert coral snake *Micrurus tschudii*, 70% of the proteins in the venom of the king cobra *Ophiophagus hannah*, (Vonk, *et al,* 2013) and the Eastern green mamba *Dendroaspis angusticeps*, (Lauridsen, *et al.,* 2016) are 3FTXs. These toxins bind post-synaptically and cause the prey animal to become flaccidly paralyzed.

Non-enzymatic neurotoxins are known as three-fingers toxins (3FTXs). They are typically found in the venoms of elapid and colubrid snakes, and they cause flaccid paralysis in snakebite victims by binding postsynaptically at the neuromuscular junctions (Barber *et al.*, 2013). Three-finger toxins come in two lengths: short-chain (3FTXs), which are composed of 57–62 residues and four disulfide bridges and include neurotoxins, cardiotoxins, cytotoxins, fasciculins, and mambalgins, and long-chain (3FTXs), which are composed of 66–74 residues and five disulfide bridges and include hannalgesin and neurotoxins. Additionally, they can exist as homo- or heterodimers that are covalent or non-covalent as well as monomers. The variety of 3FTX isoforms mentioned above is a

direct result of a complex evolutionary history, in which ancestral 3FTXs have evolved at accelerated rates of molecular evolution and frequent gene duplication. In particular, these processes are linked to the evolution of the high-pressure hollow-fanged venom delivery system found in elapid snakes. These processes are generally analogous to those underlying the evolution of the other toxin families discussed above. Examples include the expansion of 3FTX loci from one in non-venomous snakes like pythons to 19 in the elapid *Ophiophagus hannah* (king cobra), and selection appears to have had a significant impact on surface-exposed amino acid residues in these paralogous elapid 3FTX genes. The result of this evolutionary history is the differential development of a large number of 3FTX isoforms, many of which frequently display significant structural variations and unique biological activities. Although many elapid snakes exhibit broad diversity of these functionally varied toxins in their venom (e.g., multiple short- and long-chain 3FTX isoforms), it remains unclear why particular functional variants are enriched in the venoms of certain elapid lineages, such as the cytotoxin-rich venoms of cobras (genus *Naja*) or the neurotoxin-rich venoms of mambas (genus *Dendroaspis*). However, the presence of taxon-specific 3FTXs in the venoms of some "colubrid" snakes, along with the pseudogenization of 3FTXs in species that no longer depend on their venom, raises the possibility that prey capture-related selection may be at least partially to blame for the variation in 3FTX representation. Despite the shared three-finger fold, the 3FTXs have diverse targets and biological activities. For example, α-neurotoxins inhibit muscle acetylcholine receptors (nAChR), ϰ-neurotoxins inhibits neuronal AChR, muscarinic toxins inhibit muscarinic receptors, fasciculins inhibit acetylcholinesterase (AChE), calciseptine modulates L-type calcium channels, cardiotoxins interact non-specifically with phospholipids, or induce insulin secretion, mambin interacts with platelet receptors, exactin inhibits Factor X, β-cardiotoxins inhibit β-adrenoreceptors, MTα inhibits α-adrenoreceptors, mambalgins inhibit ASIC channels, Tx7335 that activates potassium channels and calliotoxin activates voltagegated sodium channels (NaV). Their toxic biological effects include necrosis through the action of cardiotoxins (cytotoxins), alteration of the cardiac rate through modulation of - and -adrenoreceptors, and altered homeostasis through inhibition of platelet aggregation and Factor X. They also inhibit AChE and ACh receptors and activate NaV1.4 and L-type calcium channels in the periphery, which results in flaccid or spastic paralysis. As evidenced by the 3FTX isoforms produced by non-front fanged snake venoms that are non-toxic to mice but highly toxic to lizards, and vice versa, 3FTXs can

also exhibit remarkable toxicities that target different classes of species in addition to their wide range of bio-activities. Some 3FTXs can cause analgesia by blocking ASIC channels, but none of them are known to cause inflammation or hyperalgesia, as is frequently described with other snake toxins. In addition, 3FTXs lack many domains necessary for their various toxic activities and are rather tiny in comparison to the other snake toxins addressed here.

The length and quantity of the disulphide bridges serve as a general basis for the structural variations amongst family members. - The two types of 3FTxs are the longer chains, which range in length from 66 to 74 residues and have five disulphide bridges (examples include hannalgesin, neurotoxins, and cytotoxins), and the shorter chains, which range in length from 57 to 62 residues and have four disulphide bridges (examples include fasciculins and mambalgins). The 3FTxs can exist as homo- or heterodimers that are covalent or non-covalent.

Despite having the same 3-finger fold, 3FTxs have different mechanisms of action. Acetylcholine receptors in muscle synapses have been found to be inhibited by neurotoxins (Changeux, 1990). For example, mambin interacts with platelet receptors (McDowell *et al.*, 1992), mambaligins inhibit ASIC channels (Diochot *et al.*, 2012), and calliotoxin activates voltage-gated sodium channels (Yang *et al.*, 2005) are just a few examples of -neurotoxins, which block acetylcholine receptors in neural synapses. It should be emphasized that none of the 3FTxs cause the hyperalgesia and inflammation that are typical of other snake toxins. The prey animal's ion channels and receptors are the primary targets of the 3Ftxs. This is due to the 3-finger fold's special aptitude and capacity to influence a variety of biological functions. Critical 3FTx segments contain certain amino acid sequences that have been found; these sequences are crucial for binding to the target locations. The initial glimpse of the fasciculin contact with the outside enzyme is shown by the interactions of Acetylcholinesterase in the prey with the three loops in the fasciculin molecule, 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) (Waqar & Batool, 2015). The interactions of neurotoxins such as neurotoxin II (NTII) from the venom of *Naja oxiana* , the Central Asian Cobra, (Mordvintsev, *et al.,* 2005) Muscarinic toxins from mamba venom (Blanchet, *et al.,* 2013) and neurotoxin b (NTb) from the venom of *O. hannah*, the King Cobra, (Peng, *et al.,* 1997) have all been thoroughly studied. Results indicate the significance of the amino acid

sequence in binding and modifying the action of the receptors. Any alteration to these sequences results in the molecule losing its neurotoxicity.

Cardiotoxins/cytotoxins

The heart muscle is attacked by these toxins, which prevents muscular contraction. This causes the heartbeat to become erratic and eventually stop. Studies have revealed that the toxins have a propensity to adhere to the muscle's surface and depolarize it. These poisons are prevalent in the venom of mambas and a few species of cobras. Other cardiotoxins cause the release of insulin or interact in an unspecific way with phospholipids (Konshina *et al*., 2017; Nguyen *et al*., 2012). B – cardiotoxins inhibit β-adrenoreceptors (Rajagopalan, *et al.,* 2007).

Cardiotoxins are extremely basic (pI>10), single-chain, small molecular weight (6.5 kDa) proteins. They display a wide range of pharmacological effect. Despite having high sequence homology with neurotoxins, the cardiotoxins exhibit surprisingly distinct characteristics. Cardiotoxins can number up to 52, and there is 90% sequence homology among them (Dufton & Hider, 1983). Cardiotoxin III (CTx III, Cytotoxin 3) is a toxin that is only found in the Taiwan Cobra (*Naja atra*) and has a length of 60 residues. Through the release of cytochrome, it can cause cells to undergo apoptosis (Yang *et al*., 2005). The crystallization of cardiotoxin VII4, which was obtained from the Mozambique spitting cobra *Naja mossambica mossambica*, demonstrated that it was a dimer with a molecular mass of 6715 Da. According to studies (Rees *et al*., 1987), the cardiotoxin inhibited nicotinic acetylcholine receptors.

Cardiotoxin-like basic proteins (CLBP) are a group of proteins that share structural similarities with cardiotoxins but differ in several ways, including the absence of the triple peptide signature (-I-D-V-) between 39 and 41, the presence of a Gln at position 17 instead of the Met residue required for activity, and the absence of the triple peptide signature (-I-D-V-) between 39 and 41 in cardiotoxins (Kumar *et al*., 1997). These molecules are now being assessed for therapeutic ability.

Applications of snake venoms

Therapeutic implications

Pharmacologically potent proteins and peptides make up snake venom. Snake venoms are more complex than those of other animals because they contain a wide variety of proteins and peptides that have a variety of pharmacological and toxicological effects.

Snake venom-based drugs

Based on the pharmacological effects produced, snake venom has been classified into haemotoxic, neurotoxic and cytotoxic venom. Only about 0.01% of snake venom has been characterized, despite the fact that snake venom is thought to be 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, and integrin antagonists possess pharmacological (Ferraz *et al* 2019).

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 (Gomes,2010) and other body fluids such as blood and bile duct in Chinese medicine (Koh & Kini, 2012) and lots of the snake venom-based drugs are available in the market and in clinical trials (Vonk *et al*., 2011).

Aggrastat® (Tirofiban), Captopril® (Enalapril), Integrilin® (Eptifibatide), and other medications based on snake venom are available on the market, and many more are in the pre-clinical or clinical trial stages (Waheed *et al*., 2012). The FDA granted approval for Captopril® in 1981, making it the first effective medication made from snake venom. The Nobel Prize winner Sir John Vane discovered this medication, a biomimetic of the bradykinin-potentiating peptide derived from the venom of the Brazilian arrowhead viper *Bothrops jararaca*, and the pharmaceutical behemoth Squibb was responsible for its commercial manufacturing. It is used to treat cardiovascular disease and hypertension because it prevents the angiotensin converting enzyme from turning angiotensin I into angiotensin II (Peng *et al*., 2005).

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 (Lazarovici, *et al*., 2019). Aggrastat, belonging to the platelet glycoprotein (GP) IIb/IIIa inhibitors and produced based on the RGD sequence (Arg-Gly-Asp) motif from snake venom disintegrins obtained from the venom of *E. carinatus* is taken to treat heart attack patients (Chen, *et al*., 1991). Based on the KGD sequence (Lys-Gly-Asp), the peptide medication Integrilin, which is used to treat acute coronary syndrome, replicates a small piece of the glycoprotein (GP) IIb/IIIa inhibitor barbourin discovered in the venom of the Southeastern pygmy rattlesnake (*Sistrurus miliarus barbouri*) (O'Shea & Tcheng, 2002). Both Aggrastat® and Integrilin® was approved for medical use by FDA in 1998.

The approved medication Defibrase®/Reptilase® (Batroxobin), based on the thrombin-like serine protease enzyme isolated from the snake venom of the two subspecies *Bothrops atrox* and *Bothrops moojeni*, is primarily used in China to treat a variety of conditions, including stroke, pulmonary embolism, deep vein thrombosis, myocardial infarction, and perioperative bleeding. Hemocoagulase®, a different medication made from *B. atrox* venom, is frequently used in plastic surgery, abdominal surgery, and human vitrectomy (Lodha, *et al*., 2011). Exanta® (Ximelagatran), a thrombin inhibitor anticoagulant produced from cobra venom, is utilized as both a blood thinner and thrombin inhibitor (Ho & Brighton, 2006).

The von Willebrand factor A1 domain's affinity for the platelet receptor glycoprotein Ibalpha (GPIbalpha) is discovered to be enhanced by the medication Botrocetin®, which was produced based on the platelet aggregating protein from the venom of *B. jararaca* (Fukuda *et al*., 2005). The blood coagulation cascade factor V activator RVV-V from *Vipera russelli* venom is being tested for destabilizing and selectively inactivating factor V in plasma (Nakayama, *et al.,* 2011). Prothrombin is activated via ecarin, a metalloprotease that was isolated from the venom of the saw-scaled viper (*E. carinatus*) (Lövgren, 2013).

Putative therapeutic substances

The venom of the Australian taipan snake, *Oxyuranus scutellatus*, contains taipoxin, a potent presynaptic neurotoxin made up of three polypeptides known as alpha, beta, and gamma subunits. Oxynor, which has pharmacological effects against wounds, is produced via trypsin breakdown of the -subunit (Lipps, 2006). Ophidia Products, Inc. subjected

Oxynor to clinical research; however, no further advancement has been documented in the literature.

Echistatin and contortrostatin were combined to create the chimeric disintegrin known as crostatin (VCN), which is present in the venom of crotalid snakes. Breast cancer models receiving intravenously administered VCN that was encapsulated in liposomes (LVCN) showed delayed tumor growth and longer animal life (Minea *et al*., 2010). Applied Integrin Sciences Inc. was conducting pre-clinical research on the medication, but no new developments have been documented in the 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 signalling pathways, thereby leading to apoptosis (Hong, *et al*., 2003). Salmosin may perhaps someday be employed as a cancer prevention tool.

A -neurotoxin called hannalgesin, which was discovered from the venom of the *O. hannah* (King cobra), has a nitric oxide- or opioid-based analgesic action. It has a stronger analgesic effect than morphine (Pu *et al*., 1995).

Snake Venom and Therapeutic Potential

Snake venom in medicine

A concoction of potent substances found in snake venom precisely and fervently target a number of crucial targets with high efficiency. All venom toxins combine their separate actions to cause deadly dysfunctions in practically any organ system. Such a toxin mimic may aid in pharmaceutically affecting a certain bodily function for the benefit of human health. These snake toxin-derived mimics are now being tested in clinical settings or are being considered for additional pharmaceutical applications, particularly in the areas of metastasis, thrombosis, and hemostasis. Due to the variety of substances it contains and their distinct biological effects, snake venom offers a significant deal of promise for use as a treatment. Cobroxin and Nyloxin, two analgesics derived from cobra venom, are used to treat severe arthritis pain and block nerve transmission similarly to morphine (Estevo-Costa, *et al*., 2018). The Malayan pitviper's arvin component works well as an anticoagulant.

Researchers are able to create new medications using venom components to treat a variety of illnesses, including Parkinson's disease, poliomyelitis, multiple sclerosis, myasthenia gravis, and multiple sclerosis (Estevo-Costa *et al.*, 2018).

Snake venom and diseases treatment

Given the large number of biologically active components found in snake venom, some of them might be helpful in the treatment of disease (McCleary & Kini, 2013). The Tunisian vipers *Cerastes cerastes* and *MacroVipera lebetina's* phospholipases type A2 (PLA2s) have been reported to have anticancer activity (Vyas *et al.*, 2013; Jain & Kumar, 2012). Because PLA2s hydrolyze phospholipids, they might interact with the surfaces of bacteria to produce new antibacterial properties. The key problem is figuring out how to get the protein to the nerve cells despite the fact that several snake venom proteins have long been known to have analgesic effect (Woolf, 2013, Osipov & Utkin, 2012).

Snake venom therapy of hepatocellular carcinoma

According to the World Health Organization (WHO), hepatocellular carcinoma (HCC) accounts for up to 90% of all liver malignancies, is the second most common cause of cancer-associated deaths, and is the fifth most common tumor globally. For the majority of advanced HCC cases, curative treatments are not available, and the prognosis is grim due to underlying cirrhosis and poor tumour response to standard chemotherapy. By boosting the survival rate by roughly three months, it only offers transient therapeutic benefit (Siegel & Zhu, 2009). In addition, systemic overexposure to SOR has caused a significant inter-individual heterogeneity in its pharmacokinetics, which has increased its toxicity (Gao *et al.*, 2012; Boudou-Rouquette *et al.*, 2012). In order to have the potential to treat cancer, it is necessary to find and create novel chemicals, such as components of snake venom (El-Magd, *et al.*, 2019). Furthermore, it is highly desirable to identify better natural, safe, and effective cancer treatment options with less toxicity and a less impact on normal cells (Shaban, *et al.*, 2018).

When two snake venoms are combined, the antiproliferative effects on liver cancer cells (HepG2) are synergistically enhanced at low dosages. Apoptotic, inflammatory, antioxidant, and cell cycle regulator gene expression was identified in this research (Mansour *et al.*, 2021).

Many poisonous animal compounds, including those from spiders, scorpions, snakes, caterpillars, centipedes, wasps, bees, toads, ants, and frogs, have demonstrated

biotechnological and pharmacological benefits against a variety of disorders, including cancer. It has been noted that the venom of snakes has a cytotoxic effect on tumor cells. This potency results from preventing cell growth and encouraging cell death by turning on the apoptotic processes. In the meantime, cytochrome-c production was enhanced by snake venom, which also treated cell membrane trigger damage and altered the expression levels of proteins that control the cell cycle.

L-amino acid oxidase (LAAO) and the intricate combinations of snake venom have an anticancer therapeutic effect by causing oxidative stress in cancer cells. According to studies, L-amino acid oxidase (LAAO) has a strong anti-tumor effect on a variety of cancer cell lines. In order to distribute hydrogen peroxide, LAAO can specifically bind to particular phospholipid compositions on the surface of cancer cells. H2O2 is created by LAAO, which also causes its cytotoxicity on the cell surface. Additionally, investigations using animal models have supported this safer impact (Mansour *et al*., 2021). In terms of cytotoxicity, when LAAO and SOR are administered together rather than separately, cell death on normal liver cells THLE-2 is reduced (Mansour *et al*., 2021). As opposed to control untreated cells, the treatment of LAAO and SV alone or in combination with SOR dramatically increased cell death and apoptosis in HepG2 cells. Furthermore, it was demonstrated that the LAAO extracted from the venom of *Ophiophagus hannah* selectively kills cancer cells by controlling the caspase activity while being non-toxic to healthy cells. Changes in the permeability of the mitochondrial membrane, which lead to Ca^{+2} overload and the release of cytochrome c, as well as apoptotic death, are effects of excessive reactive oxygen species (ROS) damage.

Therapeutic effects of snake venom on rheumatoid

The immune system predominantly targets healthy synovial joint tissue in the chronic, systemic autoimmune illness known as rheumatoid arthritis (RA) (NIH). Between 0.5% and 1.0% of people in the industrialized world are afflicted by the illness, which is a major contributor to disability. The gradual deterioration and inflammation of synovial joints, most frequently in the wrist, knee, proximal interphalangeal, and metacarpophalangeal joints, is the main feature of RA. In addition to general symptoms including fevers, exhaustion, weight loss, and discomfort, articular signs include symmetric joint swelling, tenderness, stiffness, and mobility limitation (Mansour, *et al*., 2021).

Rheumatoid arthritis and pain control have both been managed using snake venom. It has been demonstrated that the venom of the families Elapidae and Viperidae has anti-inflammatory and analgesic properties. By lowering pro-inflammatory cytokine levels and raising anti-inflammatory cytokine levels, snake venom exerts anti-inflammatory effects. Additionally, by acting as a (tumor necrosis factor alpha), TNF-alpha blocker and by preventing the development of fibroblast-like synoviocytes, snake venom can lessen structural damage from protracted inflammation. The cholinergic and opioidergic systems are involved in the modulation of snake venom pain in murine pain models, according to Wolf (2013). The results of analgesic studies involving the cholinergic system showed that not only do the effects of snake venom resemble those of morphine, but also that there are no withdrawal symptoms after venom delivery is ceased. These findings indicate tremendous promise for a non-addictive analgesic that might be utilized to treat rheumatoid arthritis sufferers' pain.

Relief of pain remained the top objective in both cohorts, despite improvements in the general health status of RA patients in Norway between 1994 and 2001, according to a study. In a different study, 88% of participants chose pain as their main area for improvement over the course of a year of therapy. Additionally, women, minorities, and people with lower levels of education tend to score higher on the pain scale, and pain is one of the main factors influencing emotional health in RA patients.

Administration of disease-modifying antirheumatic medications (DMARDs), which work peripherally to lower the inflammatory response and the pain it causes, is one of the principal treatments for pain in RA patients. Non-steroidal anti-inflammatory medicines (NSAIDs), like ibuprofen and naproxen, are additionally frequently recommended to patients as a way to alleviate their discomfort. To further reduce pain, these drugs can be combined with over-the-counter medicines like acetaminophen. Weak opioids are a possibility if NSAID and acetaminophen therapy have failed to bring relief. Treatment of the underlying inflammation that underlies the symptoms is now the main emphasis of RA therapies rather than symptom management. TNF inhibitors are used to prevent the function of tumour necrosis factor (a cytokine that promotes inflammation), which is one of the biologic disease-modifying medications that work to lower immunological responses in the body. Similar to Abatacept, Tocilluzumab suppresses the action of IL-6, another proinflammatory cytokine, but Abatacept protects T cells from becoming overactive (Osipov & Utkin, 2012).

The administration of snake venom has been found to decrease mechanical and thermal hyperalgesia in a number of mouse models. A reduction in inflammation brought on by snake venom may benefit the processing of central pain as well as central pain and sensitization. In terms of treating rheumatoid arthritis, the effects of snake venom from elapids and vipers on cholinergic and opioidergic processes of pain are undoubtedly the most promising. In one study, it was shown that snake venom, which acts on cholinergic receptors to cause analgesia, is just as potent as morphine and has a longer-lasting impact. Several research have used the anti-inflammatory and anti-arthritic effects of elapid venom or its specific components in murine arthritis models, particularly the species *Naja kaouthia* and *NajaNaja*. Observed how NN-32, a cytotoxic protein from *N. naja* venom, affected rats with arthritis. When compared to non-arthritic control rats, it was discovered that arthritic rats had significantly higher levels of the inflammatory cytokines TNF-, IL-17, and cytokine-induced neutrophil chemoattractant 1 (CINC-1, a rat cytokine (homolog of IL-8) with hyperalgesic properties). However, NN-32 treatment significantly reduced these cytokine levels. In a different study, the same researchers discovered that adjuvant-induced arthritis in rats reduced IL-10 levels, but that *N. kaouthia* venom treatment considerably increased the levels. (Osipov & Utkin, 2012).

Cobratoxin, a neurotoxin derived from a *Naja cobra*, had similar effects on arthritis-induced rats produced by complete Freund's adjuvant (CFA). The serum levels of (tumour necrosis factor) TNF-, IL-1, and IL-2 were higher in the arthritis-affected rats, but IL-10 levels were lower. Rats treated with cobratoxin showed decreased levels of proinflammatory cytokines and a reversal of the CFA-induced decline in IL-10. Similar outcomes were obtained using neurotoxin-NNA, a different peptide from *N. Naja atra*: In rat models of inflammation, the peptide treatment resulted in a dose-dependent reduction in TNF- and IL-1 levels. These investigations increase the body of proof that cobra venom can control the release of inflammatory cytokines in RA, which in turn lessens inflammatory discomfort (Minea *et al.*, 2010).

Compared dexamethasone, a corticosteroid that reduces inflammation, and the effects of cobratoxin from *N. naja atra*. This showed that dexamethasone had a stronger inhibitory effect on the long-term inflammatory process (seen by a decrease in cytokines IL-6, TNF-, and IL-1) in both the cobrotoxin- and dexamethasone-treated arthritic rats. The levels' preservation implies that CTX taken orally has anti-inflammatory capabilities by lowering pro-inflammatory cytokine levels while preserving pro-inflammatory cytokine

levels. Rats given CTX demonstrated marginally stronger analgesic and anti-inflammatory effects, indicating that venomous substances may have the ability to act as NSAIDs.

Therapeutic potential of snake venom on cancer

The protein peptides and enzymes in snake venom bind to the membranes of cancer cells and inhibit their migration and proliferation, which is how they exert their anti-cancer effects.

Uncontrolled cell division, cell transformation, escape from apoptosis, invasion, angiogenesis, and metastasis are characteristics of cancer. The most significant mechanism of many anticancer drugs is the induction of apoptosis. Since snake integrins are crucial for cell adhesion, cell migration, tissue organization, cell proliferation, hemostasis, and inflammatory reactions, researchers are looking into them to create new cancer-fighting medications. Apoptosis inducers are now recognized as essential elements in the treatment of cancer because they demonstrate control over the size and number of tumor cells when they are activated.

Platelet function and cytotoxicity were affected by the isolation and purification of L-amino acid oxidases (LAAOs) from *Bothrops leucurus* (Bl-LAAO) and cobra. The suppression of thymidine incorporation and a contact with DNA may be involved in the mechanism of action of this enzyme. Additionally, some tumor cell lines were discovered to be vulnerable to lytic activity and synthesized peptide. Additionally, NN-32 demonstrated cytotoxicity against EAC cells, prolonged the lives of EAC-inoculated mice, and decreased the size and weight of solid tumors. Proapoptotic protein levels were raised by NN-32. On rabbits, the cytotoxin from the venom of the Chinese cobra (*N. Naja atra*) was examined for its pharmacokinetic effects. An enzyme-linked immunosorbent test using biotinavidin to measure cytotoxin levels in plasma was used.

Okinawa Habu apoxin protein-1 (OHAP-1) was extracted from the venom of the animal and tested for its toxicity. In this study, OHAP-1 was able to cause certain glioma cells to commit suicide. Additionally, OHAP-1's ability to induce apoptosis in malignant glioma cells may be due to the production of intracellular ROS and the expression of the p53 protein. The Hep2 tumor volume decreased as a result of antitumor activity involving snake venom (*Lapemis curtus*), which is regarded as a significant indicator of decreased tumor burden. Cardiotoxin III (CTX III), which was extracted from the venom of *N. Naja atra*, was found to have anticancer properties. A galactoside-binding lectin that was isolated

from the venom of B. leucurus exhibited anti-tumor potential as well as cytotoxicity and hemolysis activities. The lectin BjcuL, purified from the venom of *Bothrops jararacussu*, was found to have cytotoxic effects on gastric cancer cells. This demonstrated that BJcuL is harmful to tumor cells, primarily through changing cell adherence and inducing apoptosis.

Anti-microbial potency of snake venom

Snake venoms were tested to look into their antibacterial properties, and the results were encouraging. Since the 1930s, trigeminal neuralgia, multiple sclerosis, rheumatoid arthritis, asthma, and polio have all been treated using cobra venom. The following antimicrobial substances have been identified from snake venom:

(i) L-amino acid oxidase (LAAO), and

(ii) phospholipase A2 (PLA2).

As the impact is eliminated in the presence of hydrogen peroxide scavengers like catalase, the LAAO antibacterial action appears to be caused by hydrogen peroxide produced by the oxidative action of the enzymes. From numerous poisonous snake species, antimicrobial peptides such as cathelicidins, nerve growth factor, and omwaprin have also been identified. The gram-positive and gram-negative bacteria, as well as *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *Klebsiella pneumoniae*, and *E. coli*, were all affected by the antibacterial actions of cobra venom LAAO. The purified L-amino acid oxidase from the venom of the *Bothrops pauloensis* snake was bactericidal. Gram-positive and Gram-negative bacterial strains were examined under the electron microscope, and the results revealed that the H2O2 created by LAO caused bacterial membrane rupture, which led to the loss of cytoplasmatic material. A substantial bacteriostasis effect on S. aureus was demonstrated by AkbuLAAO, a L-amino acid oxidase derived from the venom of the *Agkistrodon blomhoffii ussurensis* snake. The primary mechanism by which LAAOs kill bacteria is by inducing oxidative stress in the target cell, which results in the disruption of the plasma membrane and cytoplasm and cell death.

Salmonella typhimurium infection is treated with cathelicidin-BF, a substance found in the venom of the snake *Bungarus fasciatus*. A family of antimicrobial peptides known as cathelicidins function as versatile effector molecules in innate immunity. The first cathelicidin antibacterial peptide found in reptiles was cathelicidin-BF, which was purified from the snake venom of *B. fasciatus* (BF). Cathelicidin-BF also successfully eliminated *S. epidermidis*. Cathelicidin-BF has demonstrated high antibacterial activity against a variety of

microorganisms and is effective against mice infected with Salmonella. Antibacterial action against *S. aureus*, *P. aeruginosa*, and drug-resistant *E. coli* is provided by cathelicidin, which is present in the venom of the *B. fasciatus*. Additionally, cathelicidin BF-30 demonstrated more potent antibacterial effects on a variety of microbes.

Anti-venom

Anti-venom usage is the exclusive method of treatment for snake envenomation. Alberte Calmette created the first anti-venom for the Indian cobra (*Naja Naja*). Antivenom is produced by immunizing mammals, such as horses, goats, and rabbits, with a specific type of snake venom and isolating the associated immunoglobulin from the blood. An immune reaction to the venom will occur in the target animal, resulting in the production of antibodies against the venom's active molecule, which can subsequently be extracted from the blood and utilized to cure envenomation. Ant venom can be divided into two categories. polyvalent and monovalent genomes (Lake, 2004).

Therapeutic Role of Anti-venom

Numerous snake venom poisons are studied and turned into medicines to treat diseases like cancer, hypertension, and thrombosis. Both experimental animals and people who have been exposed to snake venom have significantly reduced blood pressure.

1. Fibrinogenolytic and Fibrinolytic Activity

Fibrinogen is removed from the bloodstream by enzymes in snake venom without being converted to fibrin. In-depth research is being done on venoms having anticoagulant characteristics for potential medical applications. Aggrastat (tirobifan), a medication used as an antiplatelet medicine (glycoprotein IIb/IIIa inhibitors), was created from a substance found in the venom of the saw-scaled viper (*Echiscarinatus*). (Markland,1998).

2. Anti-cancer Activity

The use of cobra venom in treating cancer in mice was the subject of research by Calmette *et al.* Inhibiting cell proliferation and causing morphological changes, venom has a strong cytotoxic and apoptogenic effect on human leukemic cells (Jain *et al.*, 2012; Debnath *et al.*, 2007).

3. Muscle Depolarization and Hemolysis Activity

In snakes of the elapid family, cytotoxic or cardiotoxin is a polypeptide with 60–70 amino acid residues that has been around for a while and has a variety of pharmacological

actions, including depolarizing muscles and hemolysis (Florino, *et al.,* 2013; Soto, *et al.*, 1998).

Side Effects of Anti-venom

i. Anaphylactic responses, which include fever, swollen eyes and face, difficulty breathing, skin redness, and skin swelling.
ii. Pyrogen reaction likely brought on by a significant amount of non-immunoglobullin proteins.
iii. Joint inflammation and lymph gland enlargement (Paul, 2011).

Conclusion

Nature has been a source of medicinal products for thousands of years, among which snake venoms form a rich source of bioactive molecules, such as peptides, proteins and enzymes with important pharmacological activities. Snake envenomation is one of the major health issues posing considerable morbidity and mortality; snake venoms have been used in the coagulation laboratory for the routine assay of coagulation factors and as reagents to study both coagulopathy and haemostasis. The original native snake venom compounds are usually unsuitable as therapeutics, interventions by medicinal chemists as well as scientists and clinicians in pharmaceutical R&D have made it possible to use the snake venom proteins as therapeutics for multiple disorders based on the available structural and functional information. Snake venoms, with their cocktail of individual components, have great potential as therapeutic agents for human diseases.

Recommendation

In modern Western civilization, the snake represents deceit and triggers both fascination and fear. However, ancient civilizations respected the snake owing to the healing power of its venom. It is becoming evident that the ancients were right, as the venom of this splendid animal is an extraordinary library of bioactive compounds that has great medicinal potential.

The survey of hundreds of studies conducted over the past 15 years reveals the diversity in the chemical composition of snake venom, which is increasingly being explored for the development of drugs. Efforts to elucidate the chemical reactivity of the principal toxins within venom is helping to increase understanding of how toxins act on their prey

targets, and how one can engineer toxin action to achieve a therapeutic goal. Furthermore, understanding of venom chemistry allows for the rational design of transition-state small-molecule analogue inhibitors for primary enzymatic toxins that are today the most promising candidates for replacing the difficult-to-manage and expensive antibody-based treatments for snakebite envenoming. The molecular recognition features of snake venom toxins are also being explored at a molecular level. As we have emphasized, these recognition motifs can be mimicked by small-molecule drugs directed to the toxin's targets, which is a promising approach for developing cheap, stable, easily scalable and orally available medicines in drug discovery.

The drugs already approved and under development derived from snake venom demonstrate that toxic bioactivity can be transformed into a therapy for the right disease. Large toxin molecules can be redesigned and reduced to their recognition motifs for oral delivery while maintaining affinity and specificity. Of the many drugs in preclinical development, mambalgins in particular reflect the contrast between their therapeutic promise (in this case, to relieve pain) and their origin from one of the most feared snakes on the planet.

In terms of the future of venom-based drug development, we assert that toxinomimicry is an exciting alternative and a complement to the use of unmodified toxins. Furthermore, computational chemistry, which is still underused in the field, can accelerate the understanding of snake venom chemistry and hence the development of new drugs. I hope that this seminar paper will inspire a new generation of scientists to explore and realize the immense potential of snake venoms.

References

Ahmed, M., Latif, N., Khan RA, Ahmad A, Rocha JBT, Mazzanti CM, et al. Enzymatic and biochemical characterization of *Bungarus sindanus* snake venom acetylcholinesterase. Journal of Venomous Animals and Toxins Including Tropical Diseases. 2012;18(2):236-243. ISSN 1678-9199. DOI: 10.1590/S1678-91992012000200014

Ahmed M, Rocha JB, Morsch VM & Schetinger MR (2009) Snake venom acetylcholinesterase. In Handbook of Venoms and Toxins of Reptiles (Mackessy S ed), pp. 207–219. CRC Press, London.

Ainsworth, S., Slagboom, J., Alomran, N., Pla, D., Alhamdi, Y., King, S. I., et al. (2018). The paraspecific neutralisation of snake venom induced coagulopathy by antivenoms. Commun. Biol. 1:34. doi: 10.1038/s42003-018-0039-1

Aird SD. Ophidian envenomation strategies and the role of purines. Toxicon. 2002;40(4):335-393. DOI: 10.1016/s0041-0101(01)00232-x

Ande SR, Fussi H, Knauer H, Murkovic M, Ghisla S, Fröhlich KU, et al. Induction of apoptosis in yeast by L-amino acid oxidase from the Malayan pit viper Calloselasma rhodostoma. Yeast. 2008;25(5):349-357. DOI: 10.1002/yea.1592

Barber CM, Isbister GK, Hodgson WC. Alpha neurotoxins. Toxicon. 2013;66:47-58. DOI: 10.1016/j. toxicon.2013.01.019

Barlow, A., Pook, C. E., Harrison, R. A., and Wuster, W. (2009). Coevolution of diet and prey-specific venom activity supports the role of selection in snake venom evolution. Proc. Biol. Sci. 276, 2443–2449. doi: 10.1098/rspb.2009.0048

Betzel C, Genov N, Rajashankar KR, Singh TP. Modulation of phospholipase A2 activity generated by molecular evolution. Cellular and Molecular Life Sciences. 1999;56(5-6):384-397. DOI: 10.1007/s000180050440

Bhavya J, Vineetha MS, Sundaram PM, Veena SM, Dhananjaya BL, More SS. Lowmolecular weight hyaluronidase from the venom of Bungarus caeruleus (Indian common krait) snake: isolation and partial characterization. Journal of Liquid Chromatography and Related Technologies. 2016;39(4):203-208. DOI: 10.1080/10826076.2016.1144203

Blanchet G, Upert G, Mourier G, Gilquin B, Gilles N, Servent D. New α-adrenergic property for synthetic MTbeta and CM-3 three-finger fold toxins from black mamba. Toxicon. 2013; 75:160-167. DOI: 10.1016/j. toxicon.2013.04.017

Bohlen, C. J., Chesler, A. T., Sharif-Naeini, R., Medzihradszky, K. F., Zhou, S., King, D., et al. (2011). A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. Nature 479, 410–414. doi: 10.1038/nature10607

Bordon KC, Perino MG, Giglio JR, Arantes EC. Isolation, enzymatic characterization and antiedematogenic activity of the first reported rattlesnake hyaluronidase from Crotalus durissus terrificus venom. Biochimie. 2012;94(12):2740-2748. ISSN 0300-9084. DOI: 10.1016/j.biochi.2012.08. 01422940594

Bottrall, J. L., Frank, M., Christopher, D. B., Michael, G. V., Peter, J. M., (2010). "Proteolytic activity of Elapid and Viperid Snake venoms and its implication to digestion

Boudou-Rouquette P, Narjoz C, Golmard JL, Thomas-Schoemann A, Mir O, Taieb F, et al. Early sorafenibinduced toxicity is associated with drug exposure and UGTIA9 genetic polymorphism in patients with solid tumors: A preliminary study. PLoS One. 2012;7:e42875

Bourne Y, Renault L, Marchot P. Crystal Structure of Snake Venom acetylcholinesterase in Complex with Inhibitory antibody Fragment Fab410 Bound at the peripheral Site. The Journal of Biological Chemistry. 2015;290(3):1522-1535. DOI: 10.1074/ jbc.M114.603902

Bucaretchi, F., Capitani, E. M., Vieira, R. J., Rodrigues, C. K., Zannin, M., Da Silva, N. J. Jr., et al. (2016). Coral snake bites (Micrurus spp.) in Brazil: a review of Literature reports. Clin. Toxicol. 54, 222–234. doi: 10.3109/15563650.2015.1135337

Casewell, N. R., Wuster, W., Vonk, F. J., Harrison, R. A., and Fry, B. G. (2013). Complex cocktails: the evolutionary novelty of venoms. Trends Ecol. Evol. 28, 219–229. doi: 10.1016/j.tree.2012.10.020

Casewell NR, Huttley GA, Wüster W (2012) Dynamic evolution of venom proteins in squamate reptiles. Nat Commun 3:1066

Chan, Y. S., Cheung, R. C. F., Xia, L. X., Wong, J. H., Ng, T. B., and Chan, W. Y. (2016). Snake venom toxins: toxicity and medicinal applications. Appl. Microbiol. Biotechnol. 100, 6165–6181. doi: 10.1007/s00253-016-7610-9

Changeux JP. The TiPS lecture. The nicotinic acetylcholine receptor: an allosteric protein prototype of ligand-gated ion channels. Trends in Pharmacological Sciences. 1990;11(12):485-492. DOI: 10.1016/ 0165-6147(90)90049-E

Chen Y, Pitzenberger SM, Garsky VM, Lumma PK, Sanyal G, Baum J. Proton NMR assignments and secondary structure of the snake venom protein echistatin. Biochemistry. 1991;30(50):11625-11636. DOI: 10.1021/ bi00114a004

Cousin X, Bon C. Acetylcholinesterase from snake venom as a model for its nerve and muscle counterpart. Journal of Natural Toxins. 1999;8(2):285-294

Debnath A, Chatterjee U, Das M, et al. Venom of Indian monocellate cobra and Russell's viper show anticancer activity in experimental models. J Ethnopharmacol 2007; 111(3):681-84

Dias, G. S., Kitano, E. S., Pagotto, A. H., Sant'anna, S. S., Rocha, M. M., Zelanis, A., et al. (2013). Individual variability in the venom proteome of juvenile Bothrops jararaca specimens. J. Proteome Res. 12, 4585–4598. doi: 10.1021/pr4007393

Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay AS, et al. Black mamba venom peptides target acid-sensing ion channels to abolish pain. Nature. 2012;490(7421): 552-555. DOI: 10.1038/nature11494

Doley R, Kini RM. Protein complexes in snake venom. Cellular and Molecular Life Sciences. 2009;66(17): 2851-2871. DOI: 10.1007/s00018-009- 0050-2

Dufton MJ, Hider RC. Classification of phospholipases A2 according to sequence. Evolutionary and pharmacological implications. European Journal of Biochemistry. 1983;137(3):545-551. DOI: 10.1111/ j.1432-1033.1983.tb07860.x

Durban, J., Juarez, P., Angulo, Y., Lomonte, B., Flores-Diaz, M., Alape-Giron, A., etal. (2011). Profiling the venom gland transcriptomes of Costa Rican snakes by 454 pyrosequencing. BMC Genom. 12:259. doi: 10.1186/1471-2164- 12-259

El-Magd MA, Mohamed Y, El-Shetry ES, Elsayed SA, Abo-Gazia M, Abdel-Aleem GA, et al. Melatonin maximizes the therapeutic potential of non-preconditioned MSCs in a DENinduced rat model of HCC. Biomedicine & Pharmacotherapy. 2019;114:108732

Estevão-Costa M-I, Sanz-Soler R, Johanningmeier B, Eble JA. Snake venom components in medicine: From the symbolic rod of Asclepius to tangible medical research and application. International Journal of Biochemistry & Cell Biology. 2018;104:94-113. DOI: 10.1016/j. biocel.2018.09.011

Eulalio CL, Rodrigues RS, Boldrini-França J, Fonseca FPP, Henrique-Silva F,HomsiBrandeburgo MI, et al. Molecular cloning of a hyaluronidase from Bothrops pauloensisvenom gland. Journal of Venomous Animals and Toxins Including Tropical Diseases. 2014;20. DOI: 10.1186/1678-9199-20-25

Fernandes LA, Calderon RG, Mendes MM, Costa TR, Grabner AN, Rodrigues VM, Et al. Snake venom L-amino acid oxidases: Trends in pharmacology and biochemistry. BioMed Research International. 2014;2014:196754. 19 pages. DOI: 10.1155/2014/196754

Ferraz, C. R., Calixto-Campos, C., Manchope, M. F., Casagrande, R., Clissa, P. B., Baldo, C., et al. (2015). Jararhagin-induced mechanical hyperalgesia depends on TNF-α, IL-1β and NFκB in mice. Toxicon 103, 119–128. doi: 10.1016/j.toxicon.2015.06.024

Ferraz Camila R, Arif A, Chunfang X, Casewell Nicholas R, Lewis Richard J, Kool J, et al. Multifunctional toxins in snake venoms and therapeutic implications: From pain to

hemorrhage and necrosis. Frontiers in Ecology and Evolution. 2019;7:218. DOI: 10.3389/fevo.2019.00218

Findrik Z, Geueke B, Hummel W, Vasic-Rǎcki D. Modelling of L-DOPA enzymatic oxidation catalyzed by L-amino acid oxidases from Crotalus adamanteus and Rhodococcus opacus. Biochemical Engineering Journal. 2006;27(3):275-286. DOI: 10.1016/j. bej.2005.08.022

Florino RS et al.Pharmacological study of new Asp49 phospholipase a (2) (Bbil-TX) isolated from forest viper venom in vertebrate neuromuscular preparations. Toxicon2013;69:191-99.

Frobert Y, Créminon C, Cousin X, Rémy MH, Chatel JM, Bon S, et al. Acetylcholinesterases from Elapidae snake venoms: biochemical, immunological and enzymatic characterization. Biochimica et Biophysica Acta. 1997;1339(2):253-267. DOI: 10.1016/s0167-4838(97)00009-5

Fry, B. G. (2005). From genome to "venome": molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. Genome Res. 15, 403–420. doi: 10.1101/gr.32 28405

Fry, B. G., Scheib, H., Van Der Weerd, L., Young, B., Mcnaughtan, J., Ramjan, S. F., et al. (2008). Evolution of an arsenal: structural and functional diversification of the venom system in the advanced snakes (Caenophidia). Mol. Cell Proteom. 7, 215–246. doi: 10.1074/mcp.M700094-MCP200

Fry, B. G., Vidal, N., Norman, J. A., Vonk, F. J., Scheib, H., Ramjan, S. F. R., et al. (2006). Early evolution of the venom system in lizards and snakes. Nature 439, 584–588. doi: 10.1038/nature04328

Fry, B. G., Wuster, W., Kini, R. M., Brusic, V., Khan, A., Venkataraman, D., et al. (2003). Molecular evolution and phylogeny of elapid snake venom three-finger toxins. J. Mol. Evol. 57, 110–129. doi: 10.1007/s00239-003-2461-2

Fukuda K, Doggett T, Laurenzi IJ, Liddington RC, Diacovo TG. The snake venom protein botrocetin acts as a biological brace to promote dysfunctional platelet aggregation. Nature Structural & Molecular Biology. 2005;12(2):152-159. DOI: 10.1038/ nsmb892

Gao J, Xie L, Yang WS, Zhang W, Gao S, Wang J, et al. Risk factors of Hepatocellular carcinoma–current status and perspectives. Asian Pacific Journal of Cancer Prevention. 2012;13:743-752

García-Osorio B, Lomonte B, Bénard-Valle M, López de León J, Román-Domínguez L, MejíaDomínguez NR, et al. Ontogenetic changes in the venom of Metlapilcoatlus nummifer, the Mexican jumping viper. Toxicon. 2020;184:204-214. DOI: 10.1016/j.toxicon.2020.06.023

Girish KS, Mohanakumari HP, Nagaraju S, Vishwanath BS, Kemparaju K. Hyaluronidase and protease activities from Indian snake venoms: Neutralization by Mimosa pudica root extract. Fitoterapia. 2004;75(3-4):378-380. ISSN 0367-326X. DOI: 10.1016/j.fitote.2004.01.006

Gopalakrishnakone P, Inagaki H, Vogel Ashis C-W, Mukherjee K, Rahmy TR, editors. Snake Venom. Dordrecht: Springer; 2016

Gomes A. Snake venom—an anti arthritis natural product. Al Ameen Journal of Medical Sciences. 2010;3:179

Goncalves-Machado, L., Pla, D., Sanz, L., Jorge, R. J. B., Leitao-De-Araujo, M., Alves, M. L. M., et al. (2016). Combined venomics, venom gland transcriptomics, bioactivities, and antivenomics of two Bothrops jararaca populations from geographic isolated

regions within the Brazilian Atlantic rainforest. J. Proteomics 135, 73–89. doi: 10.1016/j.jprot.2015.04.029

Grant GA, Chiappinelli VA. Kappabungarotoxin: Complete amino acid sequence of a neuronal nicotinic receptor probe. Biochemistry. 1985;24(6):1532-1537. DOI: 10.1021/ bi00327a036

Gubensek, F., Sket, D., Turk, V., and Lebez, D. (1974). Fractionation of Viperaammodytes venom and seasonal variation of its composition. Toxicon 12, 167–171. doi: 10.1016/0041-0101(74)90241-4

Gutierrez, J. M., and Rucavado, A. (2000). Snake venom metalloproteinases: their role in the pathogenesis of local tissue damage. Biochimie 82, 841–850. doi: 10.1016/S0300-9084(00)01163-9

Gutierrez, J. M., Calvete, J. J., Habib, A. G., Harrison, R. A., Williams, D. J., and Warrell, D. A. (2017). Snakebite envenoming. Nat. Rev. Dis. Primers 3:17079. doi: 10.1038/nrdp.2017.79

Gutierrez, J. M., Rucavado, A., Escalante, T., and Diaz, C. (2005). Hemorrhage Induced by snake venom metalloproteinases: biochemical and biophysical mechanisms involved in microvessel damage. Toxicon 45, 997–1011. doi: 10.1016/j.toxicon.2005.02.029

Harris, J. B., and Scott-Davey, T. (2013). Secreted phospholipases A2 of snake venoms:Effects on the peripheral neuromuscular system with comments on the role of phospholipases A2 in disorders of the CNS and their uses in industry.Toxins 5, 2533–2571. doi: 10.3390/toxins5122533

Harrison, R. A., Casewell, N. R., Ainsworth, S. A., and Lalloo, D. G. (2019). The time is now: a call for action to translate recent momentum on tackling tropical snakebite into sustained benefit for victims. Trans. R. Soc. Trop. Med. Hyg. doi: 10.1093/trstmh/try134. [Epub ahead of print].

Harrison RA, Hargreaves A, Wagstaff SC, Faragher B, Lalloo DG. Snake envenoming:A disease of poverty. PLoS Neglected Tropical Diseases. 2009;3(12):e569. DOI: 10.1371/ journal.pntd.0000569

Hassan F, El-Hawary MF, El-Ghazawy A. Acid and alkaline phosphomonoesterases in Egyptian snake venoms. Zeitschrift für Ernährungswissenschaft. 1981;20(1): 44-54. DOI: 10.1007/BF02027957

Hifumi, T., Sakai, A., Kondo, Y., Yamamoto, A., Morine, N., Ato, M., et al. (2015). Venomous snake bites: clinical diagnosis and treatment. J. Intensive Care 3:16. doi: 10.1186/s40560-015-0081-8

Ho SJ, Brighton TA. Ximelagatran: Direct thrombin inhibitor. Vascular Health and Risk Management. 2006;2(1):49-58. DOI: 10.2147/ vhrm.2006.2.1.49

Hodgson, Wayne C.; Wickramaratna, Janith C. (2002). "In vitro neuromuscular activity of snake venoms" (PDF). Clinical and Experimental Pharmacology and Physiology. 29 (9): 807- 814.doi:10.1046/j.1440-1681.2002.03740.x. PMID 12165047.

Jain D, Kumar S. Snake venom: A potent anticancer agent. Asian Pacific Journal of Cancer Prevention. 2012;13(10):4855-4860. DOI: 10.7314/ apjcp.2012.13.10.4855

Jayawardana, S., Gnanathasan, A., Arambepola, C., and Chang, T. (2016). Chronic musculoskeletal disabilities following snake envenoming in Sri Lanka: a population-based study. PLoS Negl. Trop. Dis. 10:e0005103. doi: 10.1371/journal.pntd.0005103

Jin H, Varner J. Integrins: roles in cancer development and astreatment targets. Br J Cancer 2004; 90(3): 561-65

Kasturiratne, A., Wickremasinghe, A. R., De Silva, N., Gunawardena, N. K., Pathmeswaran, A., Premaratna, R., et al. (2008). The global burden of snakebite: a

literature analysis and modelling based on regional estimates of envenomingand deaths. PLoS Med.5:e218.doi10.1371/journal.pmed.0050218

Kessler P, Marchot P, Silva M, Servent D. The three-finger toxin fold: A multifunctional structural scaffold able to modulate cholinergic functions. Journal of Neurochemistry. 2017;142(Suppl. 2):7-18. DOI: 10.1111/ jnc.13975

Kini RM, Koh CY. Metalloproteases affecting blood coagulation, fibrinolysis and platelet aggregation from snake venoms: definition and nomenclature of interaction sites. Toxins. 2016; 8(10):E284. DOI: 10.3390/toxins 8100284

Kini RM. Manjunatha excitement ahead: Structure, function and mechanism of snake venom phospholipase A2 enzymes Toxicon. Toxicon. 2003;42(8):827-840. DOI: 10.1016/j.toxicon.2003.11.002

Kleggetveit, I. P., Skulberg, P. K., and Jorum, E. (2016). Complex regional pain syndrome following viper-bite. Scand. J. Pain 10, 15–18. doi: 10.1016/j.sjpain.2015.07.005

Koh CY, Kini RM. From snake venom toxins to therapeutics-- cardiovascular examples. Toxicon. 2012;59(4):497-506. DOI: 10.1016/j. toxicon.2011.03.017

Konshina AG, Krylov NA, Efremov RG. Cardiotoxins: Functional role of local conformational changes. Journal of Chemical Information and Modeling. 2017;57(11):2799-2810. DOI: 10.1021/acs.jcim.7b00395

Kumar TKS, Jayaraman G, Lee CS, Arunkumar AI, Sivaraman T, Samuel D, et al. Snake venom cardiotoxinsstructure, dynamics, function and folding. Journal of Biomolecular Structure & Dynamics. 1997;15(3):431-463. DOI: 10.1080/07391102.1997.10508957

Lake S. Pit vipers: friends or foe? Achieves of the cold-blooded news 2004; 32(4).

Lauridsen LP, Laustsen AH, Lomonte B, Gutiérrez JM. Toxicovenomics and antivenom profiling of the eastern green mamba snake (Dendroaspis angusticeps) (PDF). Journal of Proteomics. 2016;136:248-261. DOI: 10.1016/j. jprot.2016.02.003

Lazarovici P, Marcinkiewicz C, Lelkes PI. From snake venom's disintegrins and C-type lectins to anti-platelet drugs. Toxins. 2019;11(5):303. DOI: 10.3390/ toxins11050303

Lee S-R, Latta J, Elliott LL. Comparative Biochemistry & Physiology. 1977;56C:193-197

Leon G et al. Immune response towards snake venoms. InflammAllergy Drug Targets 2011; 10(5):381-98.

Lodha A, Kamaluddeen M, Akierman A, Amin H. Role of hemocoagulase in pulmonary hemorrhage in preterm infants: A systematic review. Indian Journal of Pediatrics. 2011;78(7):838-844. DOI: 10.1007/s12098-010-0326-4

Lövgren A. Recombinant snake venom prothrombin activators. Bioengineered. 2013;4(3):153-157. DOI: 10.4161/bioe.22676

Lynch, V. J. (2007). Inventing an arsenal: adaptive evolution and neofunctionalization of snake venom phospholipase A2 genes. BMC Evol. Biol. 7:2. doi: 10.1186/1471-2148-7-2

Mahmood, A.J., Rashida, Q. and Syed M.A.Enzymatic activities of some snake venoms from families Elapidae and Viperidae Pakistan. Journal of Pharmaceutical Sciences. 1996;9(1):37-41

Mamede, C. C., De Sousa, B. B., Pereira, D. F., Matias, M. S., De Queiroz, M. R., De Morais, N. C., et al. (2016). Comparative analysis of local effects caused by Bothrops alternatus and Bothrops moojeni snake venoms: enzymatic contributions and inflammatory modulations. Toxicon 117, 37–45. doi: 10.1016/j.toxicon.2016.03.006

Markland FS. Snake venom fibrigenolytic and fibrinolytic enzymes: An updated inventory.Thrombhaemost 1998; 79:668-74

Marchot P, Bourne Y, Prowse CN, Bougis PE, Taylor P. Inhibition of mouse acetylcholinesterase by fasciculin: crystal structure of the complex and mutagenesis of fasciculin. Toxicon. 1998;36(11):1613-1622. DOI: 10.1016/ S0041-0101(98)00154-8

Mattison, Chris (2007). The New Encyclopedia of Snakes. New Jersey, USA (first published in the UK): Princeton University Press (Princeton and Oxford) first published in Blandford. p. 117. ISBN 0-691- 13295". Journal of Venom Research. 1 (3): 18–28. PMC 3086185 . PMID 21544178

Menaldo, D. L., Bernardes, C. P., Pereira, J. C., Silveira, D. S., Mamede, C. C., Stanziola, L., et al. (2013). Effects of two serine proteases from Bothrops pirajai snake venom on the complement system and the inflammatory response. Int. Immunopharmacol. 15, 764–771. doi: 10.1016/j.intimp. 2013.02.023

Menezes, M. C., Furtado, M. F., Travaglia-Cardoso, S. R., Camargo, A. C., and Serrano, S. M. (2006). Sex-based individual variation of snake venom proteome among eighteen Bothrops jararaca siblings. Toxicon 47, 304–312. doi: 10.1016/j.toxicon.2005.11.007

McCleary RJ, Kini RM. Nonenzymatic proteins from snake venoms: A gold mine of pharmacological tools and drug leads. Toxicon. 2013;62:56-74. DOI: 10.1016/j.toxicon.2012.09.008

McDowell RS, Dennis MS, Louie A, Shuster M, Mulkerrin MG, Lazarus RA. Mambin, a potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor structurally related to the short neurotoxins. Biochemistry. 1992;31(20):4766-4772. DOI: 10.1021/ bi00135a004

Mordvintsev DY, Polyak YL, Levtsova OV, Tourleigh YV, Kasheverov IE, Shaitan KV, et al. A model for short α-neurotoxin bound to nicotinic acetylcholine receptor from Torpedo californica: comparison with long-chain α-neurotoxins and α-conotoxins. Computational Biology and Chemistry. 2005;29(6):398-411. DOI: 10.1016/j.compbiolchem. 2005.08.007

Munawar, A., Ali, S. A., Akrem, A., and Betzel, C. (2018). Snake venom peptides: tools of biodiscovery. Toxins 10:E474. doi: 10.3390/toxins10110474Nakayama D, Ben Ammar Y, Miyata T, Takeda S. Structural basis of coagulation factor V recognition for cleavage by RVV-V. FEBS Letters. 2011;585(19):3020-3025. DOI: 10.1016/j. febslet.2011.08.022

Nguyen TT, Folch B, Létourneau M, Vaudry D, Truong NH, Doucet N, et al. Cardiotoxin-I: an unexpectedly potent insulinotropic agent. Chembiochem. 2012;13(12):1805-1812. DOI: 10.1002/ cbic.201200081

Ogawa Y, Kanai-Azuma M, Akimoto Y, Kawakami H, Yanoshita R. Exosome-like vesicles in Gloydius blomhoffii blomhoffii venom. Toxicon. 2008;51(6):984-993. ISSN 0041-0101. DOI: 10.1016/j.toxicon.2008.02.003

O'Shea JC, Tcheng JE. Eptifibatide: A potent inhibitor of the platelet receptor Integrin glycoprotein IIb/IIIa. Expert Opinion on Pharmacotherapy. 2002;3(8):1199-1210. DOI: 10.1517/ 14656566.3.8.1199

Osipov A, Utkin Y. Effects of snake venom polypeptides on central nervous system. Central Nervous System Agents in Medicinal Chemistry. 2012;12(4):315- 328. DOI: 10.2174/187152412803760618

Paik WK, Kim S. pH-substrate relationship of l-amino acidoxidases from snakevenomandrat kidney. Biochimica et Biophysica Acta. 1965;96(1):66-74. DOI: 10.1016/ 0005-2787(65)90610-6

Paul R (2011). Snake bite, snake venom,anti-venom and herbal antidote. A review. Indian J Red Ayur pharm.2(4):1060-67.

Peng H, Carretero OA, Vuljaj N, Liao TD, Motivala A, Peterson EL, et al. Angiotensin-converting enzyme inhibitors: A new mechanism of action. Circulation. 2005;112(16):2436-2445. DOI: 10.1161/CIRCULATIONAHA. 104.528695

Peng SS, Kumar TK, Jayaraman G, Chang CC, Yu C. Solution structure of toxin b, a long neurotoxin from the venom of the king cobra (Ophiophagus hannah). The Journal of Biological Chemistry. 1997;272(12):7817-7823. DOI: 10.1074/jbc.272.12.7817

Perry BW, Card DC, Mcglothlin JW, Pasquesi GIM, Adams RH, Schield DR, et al. Molecular adaptations for sensing and securing prey and insight into amniote genome diversity from the garter snake genome. Genome Biology and Evolution. 2018;10(8):2110-2129. DOI: 10.1093/gbe/evy157

Perry, B. W., Card, D. C., Mcglothlin, J. W., Pasquesi, G. I. M., Adams, R. H., Schield, D. R., et al. (2018). Molecular adaptations for sensing and securing prey and insight into amniote genome diversity from the garter snake genome. Genome Biol. Evol. 10, 2110–2129. doi: 10.1093/gbe/evy157

Raba R, Aaviksaar A, Raba M, Siigur J. Cobra venom acetylcholinesterase. Purification and molecular properties. European Journal of Biochemistry. 1979;96(1):151-158. DOI: 10.1111/j.1432-1033.1979.tb13024.x

Rajagopalan N, Pung YF, Zhu YZ, Wong PT, Kumar PP, Kini RM. Betacardiotoxin: a new three-finger toxin from Ophiophagus hannah (king cobra) venom with β-blocker activity. The FASEB Journal. 2007;21(13):3685-3695. DOI: 10.1096/fj.07-8658com

Rees B, Samama JP, Thierry JC, Gilibertt M, Fischert J, Schweitzt H, et al. Crystal structure of a snake venom cardiotoxin. Proceedings of the National Academy of Sciences of the United States of America. 1987;84:3132-3136

Reyes-Velasco J, Card DC, Andrew AL, Shaney KJ, Adams RH, Schield DR, et al. Expression of venom gene homologs in diverse python tissues suggests a new model for the evolution of snake venom. Molecular Biology and Evolution. 2015;32(1):173-183. DOI: 10.1093/molbev/msu294

Rivel, M., Solano, D., Herrera, M., Vargas, M., Villalta, M., Segura, A., et al. (2016). Pathogenesis of dermonecrosis induced by venom of the spitting cobra, Naja nigricollis: an experimental study in mice. Toxicon 119, 171–179. doi: 10.1016/j.toxicon.2016.06.006

Rosenberry TL, Johnson JL, Cusack B, Thomas JL, Emani S, Venkatasubban KS. Interactions between the peripheral site and the acylation site in acetylcholinesterase. Chemico-Biological Interactions. 2005;157-158:181-189. DOI: 10.1016/j. cbi.2005.10.027

Sanz L, Pla D, Pérez A, Rodríguez Y, Zavaleta A, Salas M, Lomonte B, Calvete JJ. Venomic analysis of the poorly studied desert coral snake, Micrurus tschudii tschudii, supports the 3FTx/PLA_2 dichotomy across Micrurus Venoms. Toxins June 2016;8(6):178. DOI: 10.3390/toxins8060178

Sawynok J. Adenosine and ATP receptors. Handbook of Experimental Pharmacology. 2007;177(177):309-328. DOI: 10.1007/978-3-540-33823-9_11Seo, Y. H., Park, M. R., and Yoo, S. H. (2014). Development of complex regionalpain syndrome after a snake bite: a case report. Korean J. Pain 27, 68–71. doi: 10.3344/kjp.2014.27.1.68

Shaban AM, Hammouda O, Abou Ghazala L, Raslan M, El-Magd MA. Ethyl acetate fraction of garlic (Allium sativum) inhibits the viability of MCF7 and HepG2 through induction of apoptosis and G2/M phase cell cycle arrest. Journal of Applied Pharmaceutical Science. 2018; 8:142-150

Shenoy PA, Nipate SS et al. Anti-snake venom activities of ethanolicextract of fruits of Piper longumL. (Piperaceae) against Russell's viper venom: characterization of piperine as active principle.J Ethnopharmacol 2013; 147(2):373-82.

Siegel AB, Zhu AX. Metabolic syndrome and hepatocellular carcinoma: Two growing epidemics with a potential link. Cancer. 2009;115:5651-5661

Slagboom, J., Kool, J., Harrison, R. A., and Casewell, N. R. (2017). Haemotoxic snake venoms: their functional activity, impact on snakebite victims and pharmaceutical promise. Br. J. Haematol. 177, 947–959. doi: 10.1111/bjh.14591

Smith CG, Vane JR. The discovery of captopril. The FASEB Journal. 2003;17(8):788-789. DOI: 10.1096/ fj.03-0093life Soares AM, Fontes MRM et al. Phospholipase A2 myotoxinsfrom Bothropssnake venoms: structure-function relationship. Curr Org Chem2004; 8(17): 1677-90.

Solis CE, Yarleque EA, et al. Purificaci'on y caracterizaci'on de la L-amino'acido oxidasa del veneno de la serpiente Bothrops brazili. Jerg'on shushupe. Revista Peruana de Biología. 1999;6:75-84

Soto JG (1988). Proteolytic, hemorrhagic and hemolytic activities of snake venoms. Toxicon. 26(9):875-82.

Stábeli RG, Marcussi S, Carlos GB, Pietro RCLR, Selistre-de-Araújo HS, Giglio JR, Et al. Platelet aggregation and antibacterial effects of an l-amino acid oxidase purified from Bothrops alternatus snake venom. Bioorganic & Medicinal Chemistry. 2004;12(11):2881- 2886. ISSN 0968-0896. DOI: 10.1016/j. bmc.2004.03.049

Tasoulis, T., and Isbister, G. K. (2017). A review and database of snake venom proteomes. Toxins 9:E290. doi: 10.3390/toxins9090290Tsetlin, V. I. (2015). Three-finger snake neurotoxins and Ly6 proteins targeting nicotinic acetylcholine receptors: pharmacological tools and endogenous modulators. Trends Pharmacol. Sci. 36, 109–123. doi: 10.1016/j.tips.2014.11.003

Trummal K, Samel M, Aaspõllu A, Tõnismägi K, Titma T, Subbi J, et al. 5'-Nucleotidase from Vipera lebetina venom. Toxicon. 2015 January; 93:155-163. DOI: 10.1016/j.toxicon. 2014.11.234

Vonk FJ, Casewell NR, Henkel CV, Heimberg AM, Jansen HJ, McCleary RJ, et al. The king cobra genome reveals dynamic gene evolution and adaptation in the snake venom system. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(51):20651-20656. DOI: 10.1073/pnas.1314702110

Vonk, F. J., Jackson, K., Doley, R., Madaras, F., Mirtschin, P. J., and Vidal, N. (2011). Snake venom: from fieldwork to the clinic: recent insights into snake biology, together with new technology allowing high-throughput screening of venom, bring new hope for drug discovery. Bioessays 33, 269–279. doi: 10.1002/bies.201000117

Vu TT, Stafford AR, Leslie BA, Kim PY, Fredenburgh JC, Weitz JI. Batroxobin binds fibrin with higher affinity and promotes clot expansion to a greater extent than thrombin. The Journal of Biological Chemistry. 2013;288(23):16862-16871. DOI: 10.1074/jbc.M113.464750

Vyas VK, Brahmbhatt K, Bhatt H, Parmar U. Therapeutic potential of snake venom in cancer therapy: Current perspectives. Asian Pacific Journal of Tropical Biomedicine. 2013;3(2):156- 162. DOI: 10.1016/S2221-1691(13) 60042-8

Wagstaff SC, Sanz L, Juárez P, Harrison RA, Calvete JJ. Combined snake venomics and venom gland transcriptomic analysis of the ocellated carpet viper, Echis ocellatus. Journal of Proteomics. 2009;71(6):609-623. ISSN 1874-3919. DOI: 10.1016/j.jprot.2008. 10.003

Wahby AF, El-Sayed ME, Mahdy HA, Salama WH, Abdel-Aty AM, Fahmy AS, et al. Egyptian horned viper Cerastes cerastes venom hyaluronidase: Purification, partial characterization and evidence for its action as a spreading factor. Toxicon. 2012;60(8):1380-1389. ISSN 0041-0101. DOI: 10.1016/j.toxicon. 2012.08.016

waheed H, Moin SF, Choudhary MI. Snake venom: From deadly toxins to Life-Saving Therapeutics. Current Medicinal Chemistry. 2017;24(17):1874-1891. DOI: 10.2174/0929867324666170605091546

Waqar M, Batool S. In silico analysis of binding of neurotoxic venom ligands with acetylcholinesterase for therapeutic use in treatment of Alzheimer's disease. Journal of Theoretical Biology. 2015;372:107-117. DOI: 10.1016/j.jtbi.2015.02.028

WHO (2010). Guidelines for the Prevention and Clinical Management of Snakebite in Africa. WHO.WHO (2018). Neglected Tropical Diseases. Available online at: http://www.who.int/neglected_diseases/en/ (accessed 2019).

Williams, D. J., Gutierrez, J. M., Calvete, J. J., Wuster, W., Ratanabanangkoon, K., Paiva, O., et al. (2011). Ending the drought: new strategies for improving the flow of affordable, effective antivenoms in Asia and Africa. J. Proteomics 74, 1735–1767. doi: 10.1016/j.jprot.2011.05.027

Woolf CJ. Pain: Morphine, metabolites, mambas, and mutations. The Lancet. Neurology. 2013;12(1):18- 20. DOI: 10.1016/S1474-4422(12) 70287-9

Yang RS, Tang C-H, Chuang W-J, Huang T-H, Peng H-C, Huang T-F, et al. Inhibition of tumor formation by snake venom disintegrin. Toxicon. 2005;45(5):661-669. ISSN 0041-0101. DOI: 10.1016/j.toxicon.2005.01.013

Yang SH, Chien CM, Lu MC, Lu YJ, Wu ZZ, Lin SR. Cardiotoxin III induces apoptosis in K562 cells through a mitochondrial-mediated pathway. Clinical and Experimental Pharmacology & Physiology. 2005;32(7):515-520. DOI: 10.1111/j.1440-1681.2005.04223.x

Zambelli, V. O., Picolo, G., Fernandes, C. A. H., Fontes, M. R. M., and Cury, Y. (2017b). Secreted phospholipases A2 from animal venoms in pain and analgesia. Toxins 9:406. doi: 10.3390/toxins9120406

Zelanis, A., Menezes, M. C., Kitano, E. S., Liberato, T., Tashima, A. K., Pinto, A. F., et al. (2016). Proteomic identification of gender molecular markers in Bothrops jararaca venom. J. Proteomics 139, 26–37. doi: 10.1016/j.jprot. 2016.02.030

Zelanis, A., and Tashima, A. K. (2014). Unraveling snake venom complexity with 'omics' approaches: challenges and perspectives. Toxicon 87, 131–134. doi: 10.1016/j.toxicon.2014.05.011

ZELLER EA. The formation of pyrophosphate from adenosine triphosphate in the presence of a snake venom. Arch Biochem. Aug 1950;28(1):138-139. PMID: 14771934

Zhang, C. C., Medzihradszky, K. F., Sanchez, E. E., Basbaum, A. I., and Julius, D. (2017). Lys49 myotoxin from the Brazilian lancehead pit viper elicits pain through regulated ATP release. Proc. Natl. Acad. Sci. U. S. A. 114, E2524–E2532. doi: 10.1073/pnas.1615484114

Zhang, Y. (2015). Why do we study animal toxins? Dongwuxue Yanjiu 36, 183–222. doi: 10.13918/j.issn.2095-8137.2015.4.183

Zychar, B. C., Dale, C. S., Demarchi, D. S., and Goncalves, L. R. (2010). Contribution of metalloproteases, serine proteases and phospholipases A2 to the inflammatory reaction induced by Bothrops jararaca crude venom in mice. Toxicon 55, 227–234. doi: 10.1016/j.toxicon.2009. 07.025