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Review on Snake Venom, Venom Chemistry, Anti-Venom and Traditional Treatment Treatment

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Abstract: Snake venom is highly modified saliva containing zootoxins which facilitates the immobilization and digestion of prey, and defends against threats. It is injected by unique fangs after a bite, and some species are also able to spit. envenomation has very high rate of mortality resulting from snakebites. From the seventeen families twenty-three plants were collected and explored for the first time for antisnake venom activity. According to scientific reports, the methanolic root extract of the medicinal plants *Vitex negundo*, *Hemidesmus indicus*, *Pluchea indica* and *Emblica officinalis* significantly neutralized the Viper and Cobra venom-induced pathophysiological changes. For the time being, four plants extracts explored (*Curcuma Aristolochia indica*, *aromatica*, *Andrograph is paniculata* and *Curcuma zeodaria*) for their inhibitory activity snake venom. *Echis carinatus*, *Ophiophagus hannah*, *Daboia russelli* and *Naja kaouthia* venom-induced lethal activity was significantly antagonized by the extracts of plant both in *in vitro* and *in vivo* studies. *So the* venom-induced coagulant, haemorrhage, defibrinogenating and PLA2 activity from *Daboia russellii* were significantly neutralized by the extracts. Precipitating bands between the plant extract and venom were not observed. The role of active constituents of plants and plant materials involved in snake venom inhibition was confirmed by this observation. Further studies are going on in our laboratory for the identification of active molecules as well as their mechanism of venom inhibition.
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1. Introduction

Snake venom is highly modified saliva containing zootoxins which facilitates the immobilization and digestion of prey, and defends against threats. It is injected by unique fangs after a bite, and some species are also able to spit (Bauchot, 1994). The glands that secrete the zootoxins are a modification of the parotid salivary gland found in other vertebrates, and are usually situated on each side of the head, below and behind the eye, and encapsulated in a muscular sheath. The glands have large alveoli in which the synthesized venom is stored before being conveyed by a duct to the base of channeled or tubular fangs through which it is ejected (Bottrall, *et al.,* 2010). Every year more than 100,000 people die due to snakebite worldwide. *Echis carinatus*, *Naja kaouthia and Naja naja* are the common snakes found throughout and a large number of deaths occur due to envenomation by these snakes. The antiserum is the only therapeutic agent available for the treatment of snakebite and does not provide enough protection against venom inducing haemorrhage, necrosis, nephrotoxicity and often produces hypersensitive reactions (Mattison, 2007). Antiserum development in animal is time consuming, expensive and requires ideal storage condition. Monovalent antiserum is not available and the health center is usually far and few in number. To overcome these drawbacks, there is a great need to search, to develop new affordable and suitable antidote against snakebite. The World Health Organization estimates that 80% of the world's population depends on traditional medicine for their primary health care needs. As long as man can remember, plants/plant materials have been used worldwide in traditional medicine for the treatment of different diseases. It is

estimated that even today approximately seventy percent of the world population rely on medicinal plants as their primary source of medicines (Hodgson, *et al.,* 2002). In the recent discoveries of the analgesic, anti-arthritis, antimicrobial and anticancer agent indicate the continuing importance of plant species in drug discovery. Currently, a large number of plants and plant materials are being screened for pharmacological activities especially those used in traditional/folk medicine against different diseases. Over the years, many attempts have been made for the development of snake venom antagonists especially from plant sources in spite of the existence of antiserum. In our earlier studies, it was showed that four of these plants, successfully neutralized the venom-induced pathophysiological changes (Bauchot, 1994).

Therefore, the aim of this seminar paper is: to briefly describe and provide compiled information on; venom, composition of venom, toxicity of venom, anti venom and plant extracts used for inhibition of venom.

2. Snake Venom

Snake venoms are secretion of venomous snake which are synthesized and which are stored in venomous gland *(Soares et al., 2004)*. The glands which secrete the zootoxin is a modification of the parotid salivary gland and are situated on each side of head below and behind the eye encapsulated in muscular sheath. The glands have large alveoli in which venom is stored before being conveyed by the duct to the tubular fangs, through which it is injected. Snake venom is a combination of many different proteins, peptides and enzymes and they are generally not dangerous when ingested. Snake venoms are complex mixture of enzymatic and toxic proteins, which include phospholipase A2 (PLA2s), myotoxins, hemorrhagic metalloproteinases and other proteolytic enzymes, coagulant components, cardiotoxins, cytotoxins and neurotoxins (*Leon et al., 2011)*. Snake venoms are cocktails of enzymes and non-enzymatic proteins used for both the immobilization and digestion of prey. The most common snake venom enzymes include acetylcholinesterases, L-amino acid oxidases, serine proteinases, metalloproteinases and phospholipases A2. Higher catalytic efficiency, thermal stability and resistance to proteolysis make these enzymes attractive models for biochemists, enzymologists and structural biologists (Ahmed. 2009). Here, we review the structures of these enzymes and describe their structure-based mechanisms of catalysis and inhibition. Some of the enzymes exist as protein complexes in the venom. Thus we also discuss the functional role of non-enzymatic subunits and the pharmacological effects of such protein complexes. The structures of inhibitor–enzyme complexes provide ideal platforms for the design of potent inhibitors which are useful in the development of prototypes and lead compounds with potential therapeutic applications (Frobert, 1997).

2.1. Role of venom for the snake

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. Biochemically, snake venoms are complex mixtures of pharmacologically active proteins and polypeptides. All of them in concert help in immobilizing the prey. A large number of protein toxins have been purified and characterized from snake venoms (Bauchot, 1994) and snake venoms typically contain from 30 to over 100 protein toxins. Some of these proteins exhibit enzymatic activities, whereas several others are non-enzymatic proteins and polypeptides. Based on their structures, they can be grouped into a small number of toxin superfamilies. The members in a single family show remarkable similarities in their primary, secondary and tertiary structures but they often exhibit distinct pharmacological effects. The most common enzymes in snake venoms are phospholipase A2s (PLA2s), serine proteinases, metalloproteinases, acetylcholinesterases (AChEs), l-amino acid oxidases, nucleotidases (5 ¢ -nucleotidases, ATPases, phosphodiesterases and DNases) and hyaluronidases. In most cases, snake venoms are the most abundant source for all these enzymes. For example, Bungarus venoms are rich in AChE (0.8% w⁄ w) (Rodríguez *et al.,* 1983).

2.2. Snake Milking Process, preservation and storage of venom

Snakes can be milked according to a regular schedule, depending on the species. The interval between milking varies among producers and ranges from every 2 or 3 weeks to every 3 months. For very dangerous species, the use of short-acting general anesthesia or moderate cooling (15°C) during milking can be considered (e.g. inhaled sevofluorane or sevoflurane, halothane or even carbon dioxide) as it reduces the risk of accidents both to the snake and to the snake-handler (Florino *et al*., 2013*)*. For the collection of venom, the snake's head is grasped between index finger and thumb, just behind the angle of the jaw, while the snake's body is held between the trunk and the arm of the snake handler (Florino *et al*., 2013*)*. By applying gentle pressure, the snake's jaws are forced open, the fangs exposed. The fangs are pushed through a plastic/parafilm

membrane hooked over the lip of a glass vessel, and venom is squeezed out. Any venom sample contaminated with blood should be rejected. After venom extraction, the fangs are carefully withdrawn from the collection vessel, while preventing damage to the mouth and dentition and avoiding the snake's impaling itself with its own fangs. After each venom milking, all materials used for milking should be sterilized with a flame, and then cooled with a draught of air before the next snake is milked. During milking, the wearing of protective clothing and a mask as well as vinyl gloves is recommended to prevent any accidents or infections (Florino *et al*., 2013*).* Snake venom can be preserved and stored in desiccators at 4˚C for further use. It was dissolved in 0.9% saline and sent, stored at 4˚C until further use. Venom concentration was expressed in terms of dry weight (Alam, and Gomes, 2003).

2.3. Type of Snake Venom

Variation in venom composition is a ubiquitous phenomenon in snakes and occurs both interspecifically and intraspecifically. Venom variation can have severe outcomes for snake bite victims by rendering the specific antibodies found in antivenoms ineffective against heterologous toxins found in different venoms Casewell *et al.,* (2012). Different species have different type's venom which depends upon its species, geographical location, its habitat, climate, age etc. There are three types of venom according to its effect viz. Haemotoxic, Cytotoxic & Neurotoxic. 1) Haemo-toxic venoms are one which affects cardiovascular system, 2) Cytotoxic venoms targets specific cellular sites and 3) Neuro-toxic venoms harm nervous system of human body. Enzymes present in snake venom hydrolyze protein and membrane components which lead to tissue necrosis and blood clotting (Jin and Varner, 2004). Snakes have fascinated mankind since prehistoric times. They are one of the few living organisms which evoke a response positive or negative when one hears a hissing or rattling sound or even a mere mention of the word 'snake' (Shenoy *et al.*, l2013). This intense fascination probably arises from the deadly effect of their venoms, which wheninjected into the victim cause a variety of physiological reactions such as paralysis, myonecrosis and often death. Snake venoms have evolved in to complex mixtures of pharmacologically active proteins and peptides that exhibit potent, lethal and debilitating effects to assist in prey capture. Their diet is very varied and includes small animals, snails, fishes, frogs, toads, lizards, chickens, mice, rats and even other snakes (Debnath *et al*., 2007).

2.4. Chemistry of venoms

Venoms contain more than 20 different compounds, mostly proteins and polypeptides (Halliday *et al.,* 2002). A complex mixture of proteins, enzymes, and various other substances with toxic and lethal properties serves to immobilize the prey animal; enzymes play an important role in the digestion of prey (Bottral *et al., 2010).* and various other substances are responsible for important but non-lethal biological effects. Some of the proteins in snake venom have very specific effects on various biological functions including blood coagulation, blood pressure regulation, and transmission of the nervous or muscular impulse, and have been developed for use as pharmacological or diagnostic tools, and even useful drugs (Bauchot, 1994). Snake venom consists of protein, enzymes, neurotoxins, coagulants, anti-coagulants and substances with cytotoxic effects. It has acidic pH. Specific gravity is 1.03 and is water soluble. Phosphodiesterase A2 causes hemolysis by lysing cell membrane of RBCs. Oxidases and proteases are used for digestion. Snake venom contains inorganic cations such as sodium, potassium, magnesium and small amount of zinc, nickel, cobalt, iron. Zinc is necessary for anticholinesterase activity. Calcium is required for activation of enzyme like phospholipase. Two major classification of toxins found in snake venom include neurotoxins and Cyto-toxin (Jain *et al*., 2005). Proteins constitute 90-95% of venom's dry weight and they are responsible for almost all of its biological effects. Among hundreds, even thousands of proteins found in venom, there are toxins, neurotoxins in particular, as well as nontoxic proteins (which also have pharmacological properties), and many enzymes, especially hydrolytic ones (Bauchot, 1994). Enzymes make-up 80-90% of viperid and 25-70% of elapid venoms: digestive hydrolases, L-amino acid oxidase, phospholipases, thrombin-like pro-coagulant, and kallikrein-like serine proteases and metalloproteinases (hemorrhagins), which damage vascular endothelium. Polypeptide toxins (molecular weight 5-10 KDa) include cytotoxins, cardiotoxins, and postsynaptic neurotoxins (such as α-bungarotoxin and α-Cobratoxin), which bind to acetylcholine receptors at neuromuscular junctions. Compounds with low molecular weight (up to 1.5 KDa) include metals, peptides, lipids, nucleosides, carbohydrates, amines, and oligopeptides, which inhibit angiotensin converting enzyme (ACE) and potentiate bradykinin (BPP). Inter- and intra-species variation in venom chemical composition is geographical and on to genic (Halliday *et al.,* 2002). Phosphodiesterases interfere with the prey's cardiac system, mainly to lower the blood pressure. Phospholipase A2 causes hemolysis by lysing the phospholipid cell membranes of red blood cells (He

et al., 2004). Amino acid oxidases and proteases are used for digestion. Amino acid oxidase also triggers some other enzymes and is responsible for the yellow colour of the venom of some species. Hyaluronidase increases tissue permeability to accelerate absorption of other enzymes into tissues. Some snake venoms carry fasciculins, like the mambas (*Dendroaspis*), which inhibit cholinesterase to make the prey lose muscle control (Bernardoni *et al*., 2014).

Structure of venom AChE:

Structurally, AChE purified from the venom of Bungarus fasciatus and other Elapidae venom exists as soluble monomers that are not associated with either anchoring proteins or cell membranes (Cousin et al., 1996). Sequence comparisons of snake venom AChE with other AChEs demonstrate that the catalytic domains of the enzymes exhibit a high level of homology (Rodrigues *et al*., 2009). Homology modeling of Bungarus fasciatus AChE. The structure is derived using scientific molecular modeling with the automated mode of homology modeling on the Swiss-Model Protein Modeller Server (Arnold *et al.,* 2006). (A) The active site pocket of the modeled enzyme, with the conserved catalytic active site residues highlighted in red and the peripheral site residues highlighted in blue. (B) The entrance to the active site gorge of the enzyme, whereby Tyr70 and Asp285 (highlighted in orange) reside in close proximity to the active and peripheral site of Torpedo AChE. These residues are replaced by methionine and lysine residues (highlighted in magenta) respectively in the Bungarus fasciatus homolog (Colletier *et al*., 2006).

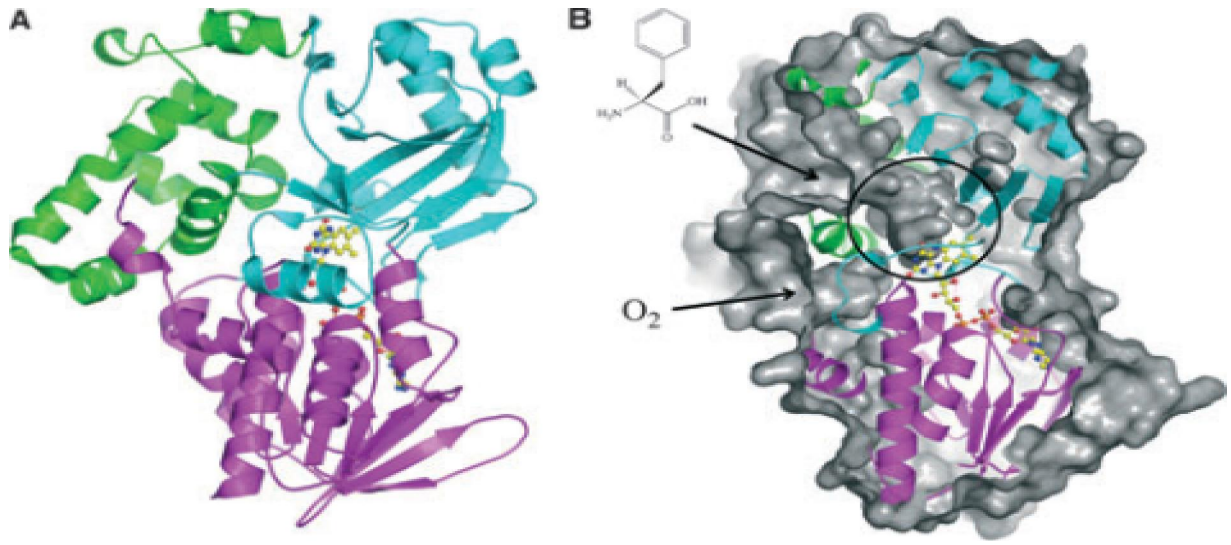

Fig. 1. The structure of L-amino acid oxidase from the snake venom of Calloselasma rhodostoma. (A) A ribbon representation Showing the three domains of the structure: magenta coloring represents the FAD binding domain, cyan represents the substrate binding domain and green represents the helical domain. (B) The accessible surface representation of the structure: the amino acid entry and the oxygen entry points are marked with arrows and the active site is circled. The FAD molecule is shown with a ball-and-stick representation. 3

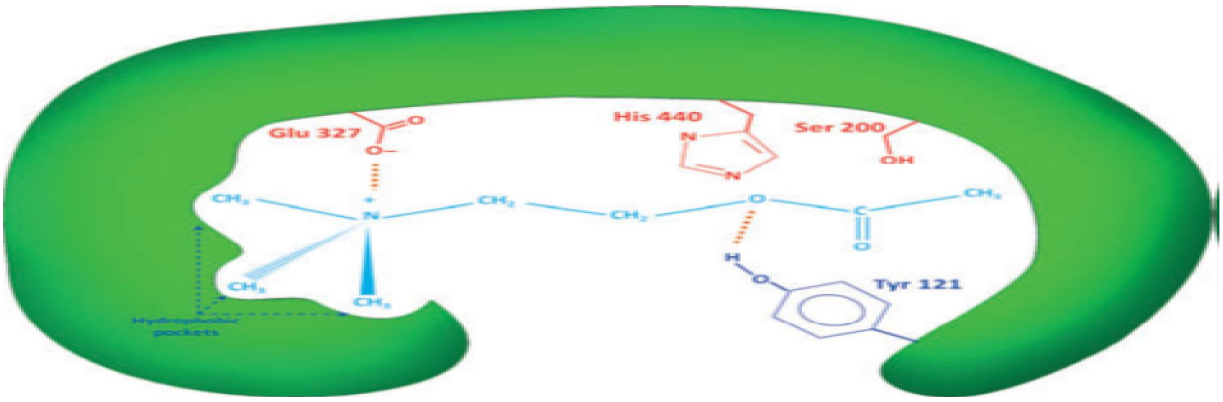

Fig. 2. Schematic representation of Torpedo AChE active site (Ahmed *et al.* [22] and Patrick et al. [240]. Residues involved in the catalytic triad are highlighted in red, while residues and partial contributions from the peripheral anionic sites are shaded in blue. 3

2.5. Toxicity of venom

Snake toxins vary greatly in their functions. Two broad classes of toxins found in snake venoms are neurotoxins (mostly found in elapids) and hemotoxins (mostly found in viperids) (Condrea *et al.,* 1964). However, there are exceptions - the venom of the black-necked spitting cobra (*Naja nigricollis*), an elapid, consists mainly of cytotoxins, while that of the Mojave rattlesnake (*Crotalus scutulatus*), a viperid, is primarily neurotoxic. There are numerous other types of toxin which both elapids and viperids may carry (Bernardoni *et al*., 2014)**.** The toxic composition of snake venom varies between species. Such variation can have major medical implications for the treatment of human snakebite victims. Venom variation is largely attributed to differences in toxin-encoding genes present in the genome or venom gland of snakes. Here, mechanisms affecting the transcription, translation, and posttranslational modification of toxins also significantly contribute to the diversity of venom protein composition (Barlow et al., 2009). Venom variation observed between related snake species is therefore the result of a complex interaction between a variety of genetic and postgenomic factors acting on toxin genes. Ultimately, this variation results in significant differences in venom-induced pathology and lethality and can undermine the efficacy of antivenom therapies used to treat human snakebite victims (Fox and Serrano, 2008).

2.5.1. Neurotoxins

The beginning of a new impulse: A) An exchange of ions across the nerve cell membrane sends a depolarizing current towards the end of the nerve cell (cell terminus). B) When the depolarizing current arrives at the nerve cell terminus, the neurotransmitter acetylcholine (ACh), which is held in vesicles, is released into the space between the two nerves (synapse). It moves across the synapse to the postsynaptic receptors. C) ACh binds to the receptors and transfers the signal to the target cell, after a short time it is destroyed by acetylcholinesterase (Patrick, 2001).

***Dendrotoxins*:** Dendrotoxins inhibit neurotransmissions by blocking the exchange of positive and negative ions across the neuronal membrane lead to no nerve impulse, thereby paralyzing the nerves. Snake example*: mambas), (*Massoulie *et al.,* 1993).

α-neurotoxins: Alpha-neurotoxins are a large group, with over 100 postsynaptic neurotoxins having been identified and sequenced.[8] α-neurotoxins attack the Nicotinic acetylcholine receptors of cholinergic neurons. They mimic the shape of the acetylcholine molecule and therefore fit into the receptors → they block the ACh flow → feeling of numbness and paralysis. Snake examples*: king cobra (Ophiophagus hannah) (known as hannahtoxin containing α-neurotoxins), (*Massoulie *et al.,* 1993) *sea snakes (Hydrophiinae) (known as erabutoxin), manybanded krait (Bungarus multicinctus) (known as α-Bungarotoxin), and cobras (Naja spp.) (known as cobratoxin)* (Patrick, 2001).

2.5.2. Cytotoxins

***Phospholipases*:** Phospholipase is an enzyme that transforms the phospholipid molecule into a lysophospholipid (soap) ==> the new molecule attracts and binds fat and ruptures cell membranes.

Snake example*: Okinawan habu (Trimeresurus flavoviridis) (*Massoulie *et al.,* 1993).

***Cardiotoxins*:** Cardiotoxins are components that are specifically toxic to the heart. They bind to particular sites on the surface of muscle cells and cause depolarisation ==> the toxin prevents muscle contraction. These toxins may cause the heart to beat irregularly or stop beating, causing death. Snake example*: mambas, and some cobra species), (*Massoulie *et al.,* 1993).

***Hemotoxins*:** Hemotoxins cause hemolysis, the destruction of red blood cells (erythrocytes), or induce blood coagulation (clotting). A common family of hemotoxins are snake venom metalloproteinases.[10] Snake example: most vipers and many cobra species. The tropical rattlesnake *Crotalus durissus* produces convulxin, a coagulant (Silman I & Sussman, 2008).

2.5.3. Role of non-enzymatic proteins

In most cases, snake venom enzymes act as monomers and exhibit optimal pharmacological properties and contribute to toxicity. At times, they form complexes with other non-enzymatic proteins to achieve higher efficiency through synergy. These non-enzymatic components play distinct functional roles in different complexes. In serine proteinase prothrombin activators (such as pseutarin C), a FVa-like non-enzymatic subunit enhances the Vmax of the prothrombin activation reaction [181], whereas in procoagulant metalloproteinases (such as RVV-X and carinactivase), two light chains of C-type lectin-like protein contribute to specificity (FX and prothrombin, respectively) and Ca2+ dependence (Morita, 2005). In ncHdPLA2s, the basic components are toxic and induce a number of pharmacological effects, but the acidic subunits are neither toxic nor enzymatically active (or possess very low catalytic activity). These non-enzymatic subunits play distinct roles in different complexes. The crotoxin subunits dissociate when the complex interacts with synaptic membranes. The toxic PLA2 binds to a specific membrane receptor while the non-toxic component remains in solution. The acidic subunit behaves as a 'chaperon' preventing a non-specific binding of the enzyme to other substrates and potentiates the toxicity (Bon *et al.,* 1979). The acidic component of viperotoxin F also potentiates the neurotoxicity of the basic subunit but reduces its enzymatic activity, while that of vipoxin plays a multifunctional role. It stabilizes the neurotoxic component of the complex preserving the toxicity for a long time, and decreases the neurotoxicity of the basic PLA2 and its catalytic activity. In b-BTx, the covalently linked proteinase inhibitor-like subunit confers the target specificity by binding to voltage-dependent potassium channels (Doley & Kini, 2009). Thus non-enzymatic components contribute significantly to the pharmacological efficiency of their respective enzymatic subunits. However, some questions are yet to be answered: why in some cases (crotoxin, viperotoxin F) does the acidic chain potentiate the toxicity but in others (vipoxin) reduce the pharmacological activity? How does the acidic subunit in vipoxin assist basic PLA2 to switch the target from the presynaptic to postsynaptic side? Do they also affect other pharmacological effects of the toxic components? Conclusions and future prospects Venoms of snakes represent a veritable source of potent pharmacologically active molecules. The primary purpose of developing such a lethal concoction of toxins was probably for prey capture and defense, and venom proteins have certainly evolved to exhibit a plethora of novel pharmacological functions with impressive specificity and functions (Doley & Kini, 2009). Higher catalytic efficiency, heat stability and resistance to proteolysis as well as abundance of snake venom enzymes compared with non-venom homologs make them attractive models for biochemists, enzymologists and structural biologists. Despite sharing similar structural scaffolds, some of these enzyme toxins exhibit multiple pharmacological functions. Thus, structure–function relationships of such enzymes pose intriguing and exciting challenges to scientists. Structural studies of the enzymes have not only contributed to our understanding of the mechanism of catalysis but also to that of their inhibition. Using structural information, highly specific, nanomolar affinity inhibitory peptides have been designed successfully for PLA2 enzymes. These encouraging initial successes will provide impetus. The inhibitors have significant importance in developing therapeutic prototypes and lead compounds for various human diseases and ailments (Doley & Kini, 2009).

3. Anti-venom

The only available treatment against snake bite is the usage of anti-venom. The first anti-venom was developed by Alberte Calmette against the Indian cobra (NajaNaja). Anti-venom is made by immunizing mammals such as horse, goat, rabbit with particular snake venom and the specific immunoglobins are isolated from the blood. The subject animal will undergo an immune response to the venom, producing antibodies against the venom's active molecule which can then be harvested from the animal's blood and used to treat envenomation. Ant venom is classified into two types. Monovalent anti venom when they are effective against a given

species venom. Polyvalent when they are effective against a range of species (Lake *et al.,* 2004).

3.1. **Stability of anti-venom**

Liquid preparations have a shelf-life of up to 3 years at 2-8 °C, and freeze-dried preparations up to 5 years, when kept in the dark at room temperature. It is highly recommended that manufacturers perform stability studies to evaluate the possibility that their preparations could be stored for a long period under non-refrigeration (for instance at 30 °C). Real-time stability tests should be performed under the expected storage conditions of the anti-venom (Lake *et al.,* 2004).

3.2. **Storage of anti-venom**

Anti-venom should be stored at a temperature within the range that assures stability, as found by stability tests. This is particularly critical for liquid formulations, which usually require storage at between 2 and 8 °C (Markland, 1998).

3.3. Therapeutic Role of Anti-venom

Many toxins from snake venom are investigated and formulated into drugs for the treatment ofconditions such as cancer, hypertension and thrombosis. Snake venom significantly lowers the blood pressure in human victims and experimental animals (Lake *et al.,* 2004).

3.3.1. Fibrinogenolytic and fibrinolytic

Snake venom enzymes remove fibrinogen from the circulation without converting it to fibrin. Venoms with anticoagulant properties are extensively studied for possible medical applications. The drug Aggrastat (tirobifan) was developed from a compound in the venom of the saw-scaled viper (Echiscarinatus), and issued as an antiplatelet drug (glycoprotein IIb/IIIa inhibitors) (Markland, 1998).

3.3.2. Cardiotonic and antiarrythmic

Malayan pit viper venom has blood thinning properties and could be effective in treating stroke patients. Gomes et al identifies a non-protein micro molecular toxin from the Indian cobra. This toxin possesses antiarrhythmic properties at microgram level (Jain *et al.,* 2012).

3.3.3. Anti-Cancer activity

According with investigated the use of cobra venom in the treatment of cancer in mice. In case of in vitro study, venom showed potent cytotoxic and apoptogenic effect on human leukemic cells (U937/K562) by reducing cell proliferation rate and produced morphological alterations (Jain *et al., 2012).*

3.3.4. Muscle depolarization & Hemolysis

Cytotoxin or Cardiotoxin are polypeptide of 60-70 amino acid residues long found in snakes of elapid family having various pharmacological effects such as depolarization of muscles, and hemolysis (Soto *et al.,* 1988).

3.4. Side effects of anti-venom

Snake venom anti-venom has the following side effects; 1) Anaphylactic reactions: such as difficulty in breathing, reddening of skin, swelling of eyes and face, fever. 2) Pyrogen reaction: probably due to the action of high concentrations of non-immunoglobulin proteins Inflammation of joints Enlargement of lymph gland (Paul *et al*., 2011).

4. Plants used for Snake bite

The plant constituents are used to neutralize the effects of snake venoms. The way of management of snake bites designed to control infection, stop pain, improve symptoms, correct imbalance, adjust immune system and boost energy for better health and quality of life (Soto *et al.,* 1988).

4.1. Aristolochia odoratissima

In the low lands south of Maracaibo Lake people drink an infusion of *Aristolochia odoratissima* leaves to treat snake bites.Toxicity studies showed that the aqueous extract of A. odoratissima did protect the mice against the lethal effects of Bothropsatrox venom. Nevertheless, protection was only observed at higher doses of venom (8 and 16 mg/kg), without modifying the values at the lower doses (Sailakshmi *et al.,* 2012).

Fig. 3: *Aristolochia odoratissima*

4.2. Tamarindus indicus

Aqueous and alcoholic extracts of dried seed powder of *Tamarindus indicus* were tested for their antioxidant and inhibitory activity of toxic enzymes like PLA2 and proteinases of Najanaja venom. The methanolic extracts of *T. Indica*seed possess compounds, which inhibit the activity of Phospholipase A2 and Proteinases of cobra venom. It may be used as an alternative treatment to serum therapy and as a rich source of potential inhibitors of toxins involved in several pathological conditions of humans and animal diseases (Sailakshmi *et al.,* 2012).

4.3. Holarrhena antidysenterica

Jain and Srivastava have reported the use of the bark against snake bite. Prusti and Behera, in an ethno-medico-botanical study of Sundargarh District,Orissa, India, have reported the roots rubbed on a stone with a few drops of water and the paste obtained is given internally and applied externally in snakebite (Jain *et al.,* 2005).

4.4. Andrographis paniculata

Paniculata plant extract has anti-venom activity against *Najanaja* venom. The leaves of A. paniculata contains andrographolide, the active constituent of which is diterpene and is responsible for ASV property by modifying the actions of proteins, and enzymes also inhibit snake venom phospholipase A2 activities (Jhon *et al.,* 2011).

Figure 4: *Andrographis paniculata*

5. Tests to Determine Anti Venom Activity

5.1. In vivo animal testing

The protection of whole animals against a dose of venom by the plant extracts is impractical now-a-days because of ethical considerations. Recently mice have been used for the testing of crude extracts. A lethal dose of the venom was mixed with the varying doses of the plant extract and injected into the animal. Later the survival rate with and without extracts was determined (Asuzu and Harvey, 2003).

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5.2. Testing using isolated organ preparations

The test consists of measurements on nerve–muscle preparations, isolated muscles and studies on blood clotting procedures. The cobra venoms that impair neuromuscular transmission are experimentally studied using nerve muscle preparations from neck of chick (biventercervicis) and abdomen of the rat (phrenic nerve hemi diaphragm). Indirect stimulation of these preparations is inhibited by the venoms. Plant extracts containing anti-venom activity may consequently reverse these inhibitory effects. This was demonstrated with *Curcuma longa* extract against the neurotoxin from *Naja najasiamensis*. Envenomization by the Carpet viper, *Echiscarinatus* causes rapid intra - arterial clotting of blood, resulting in internal haemorrhage due to depletion of fibrinogen. *Mucuna pruriens* (Naikurana**;** Leguminosae) increased the clotting time of blood induced by *E. carinatus* venom (Asuzu and Harvey, 2003).

5.3. Tests using Enzymes

The enzyme based assays were used for enzyme inhibition or enzyme activation of large numbers of plant extracts.The potassium salt of gymnemic acid isolated from *Gymnemasylvestre* (Asclepiadaceae) inhibits ATPase from cobra and viper venom. Inhibition occurs due to competitive binding between gymnemate and ATP (Prusti and Behera, 2007).

6. Traditional Treatments

The World Health Organization estimates that 80% of the world's population depends on traditional medicine for their primary health care needs (Kadiyala *et al*, 2011). Methods of traditional treatment of snake bite, although of questionable efficacy and perhaps even harmful, are nonetheless relevant. Plants used to treat snakebites in Trinidad and Tobago are made into tinctures with alcohol or olive oil and kept in rum flasks called 'snake bottles'. Snake bottles contain several different plants and/or insects. The plants used include the vine called monkey ladder (*Bauhinia cumanensis* or *Bauhinia excisa*, Fabaceae) which is pounded and put on the

bite. Alternatively a tincture is made with a piece of theAntivenom snakebite treatment must be matched as the type of envenomation that has occurred. In the Americas, polyvalent antivenoms are available that are effective against the bites of most pit vipers. Crofab is the antivenom developed to treat the bite of North American pit-vipers (Kini & Chan, 1999). These are not effective against coral snake envenomation, which requires a specific antivenom to their neurotoxic venom (Bernardoni *et al.,* 2014)**.**

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