VENOMOUS SNAKES

Snakes are highly evolved reptiles belonging to the Phylum: Chordata, Order: Squamata and Suborder: Serpentes. They are widely distributed throughout the world except Antarctica (Deoras, 1965). They are common in tropical and subtropical regions, but are rarely found at high altitudes while their number increases in humid regions (Russell, 1980). Of the 3000 species of snakes identified, approximately 500 are considered to be poisonous (Russell and Brodie, 1974; Philip, 1994; Fry, 2005). As per recent update of classification based on morphological characteristics including arrangement of scales, dentition, osteology, myology and sensory organs etc., venomous snakes have been classified in to three families (Wuster and Harvay, 1996; Wuster et al., 1997; Fry, 2005).

Viperidae: This includes the Russell's viper, saw-scaled viper, puff adder, gaboon viper, horned viper of Sahara, European vipers, rattlesnakes, copperheads, water moccasins of North America, bushmaster of South America and pit vipers of Asia.

Elapidae: This includes cobras, coral snakes, kraits and mambas.

Hydrophidae: This includes all sea snakes.

The family Viperidae is further classified into two-sub families, Viperinae and Crotalinae. The Viperinae subfamily includes Russell's viper, saw-scaled viper, puff adder, goboon viper, horned viper of Sahara and European vipers. The Crotalinae subfamily includes rattlesnakes, copper head, water moccasins and pit vipers of Asia. Deoras in 1965 has listed about 216 species of snakes out of which 52 are poisonous in the Indian subcontinent. However, "Romulus Whitaker and Ashok Captain (2004) have now provided a comprehensive list of 275 snakes found in various parts of Indian subcontinent. Among venomous snakes, mainly four pose threat to human beings as they are found in the vicinity of human settlement and are endemic, especially in rural areas, which are agricultural and have rats in abundance. The four major venomous snakes are called `Big Four` *Naja naja* (cobra), *Daboia/Vipera russellii* (Russell's viper), *Echis carinatus* (saw-scaled viper) and *Bungarus caeruleus* (krait). According to the WHO

guidelines, *Ophiophagus hannah* (king cobra) is also included among the endemic Indian venomous snakes. The distribution of these big four snakes is given in table 1.1. Apart from these snakes the other venomous snakes that strike human beings are Banded krait (*Bungarus fasciatus*) and Indian monocled cobra (*Naja kaouthia*).

SNAKE VENOM

Snake venom is a specialized exocrine secretion of the oral glands called the Jacobson's glands, which are located along the upper Jaw. In snakes, venom is an evolutionary adaptation to immobilizing, killing and initiating the digestion of the prey. It may also serve as a principle defensive weapon for protecting the snakes against the predators and aggressors. Snake venoms are extremely a complex mixture of biologically active but target specific enzymatic and non-enzymatic protein and peptide toxins. To provide victims with a lethal hit, venom glands synthesize, stores and secretes mixture of predominantly proteins/peptide components with different structures and functions as either the active/inactive precursor forms into the site of their bite. The precursor forms of components are activated by a special mechanism after the secretion. In addition to protein /peptide toxins, snake venom is also composed of several inorganic and organic compounds such as Ca, Cu, Fe, K, Na, P and Zn, free amino acids, citrates, nucleosides, purines, carbohydrates and lipids (Arid, 2002). The biological role of each of the metal ion is not clear, however, they may be required for the catalytic function of venom enzymes or they may be essential for stabilizing certain venom enzymes/proteins.

The venom components seem to be fairly common and similar to one another within each family of snakes. The target of snake venom toxins vary, while the Elapid and Hydrophid venoms have mainly neurotoxic effects and hemorrhagic and myonecrotic toxins are generally found in the venoms of Viperid and Crotalid snakes, but are basically different depending on each snake species (Tu, 1991). However, snake venoms exhibit marked variations in their potency and extent of induction of toxic properties. The variability of venom composition has been considered at several levels: Interfamily,

intergenus, interspecies, intersubspecies and intraspecies. While intraspecies variability may be due to geographical distribution, seasonal and age dependent change, diet and variations due to sexual dimorphism (Chippaux et al., 1991; Daltry et al., 1996; Sasa, 1999; Shashidhara murthy et al., 2002).

The biologically active protein and peptide toxins in snake venoms can be either enzymatic or non-enzymatic in property. Earlier investigators tried to explain all the biological activities of snake venoms based on the presence of enzymes or combination of enzymes. However, the initial contributions of several researchers (Weiland and Konz, 1936; Slotta and Fracnkel-Conrat, 1938; Ghosh et al., 1941), it becomes evident that there are several non-enzymatic proteins exist in snake venoms and have been shown to cause neurotoxicity (Larsen and Wolf, 1968; Sato et al., 1969), myotoxicity (Ownby et al., 1976; Chang, 1979; Lomonte and Gutierrez, 1989), cardiotoxicity and platelet aggregation (Kini et al., 1988). Nerve growth factors (Oda et al., 1989; Kostiza and Meier, 1996) and bradykinin potentiating peptides are also reported from snake venoms (Ondetti et al., 1971; Aird, 2002). To name a few non-enzymatic toxins, myotoxin I from *Crotalus viridis concolor* (Engle et al., 1983), peptide C from *Crotalus viridis helleri* (Maeda et al., 1978), dendrotoxin (DTX) from *Dendroaspis angusticeps* (Harvey and Karlsson, 1980), cardiotoxin from *Naja nigricollis* (Kini et al., 1987, 1988), trypsin inhibitor (TI) from *Daboia russellii* (Jayanthi and Gowda, 1990), phospholipase A inhibitor (NN-I_3) from *Naja naja (*Rudrammaji, 1994) venoms have been studied.

Some of the enzymes found in snake venoms are as follows hyaluronidase, metalloprotese, serine protease, aspartic/thiol protease, phospholipase A_2, L-amino acid oxidase, 5'-nucleotidase, phosphodiesterase, arginine ester hydrolase, kininogenase, acetylcholinesterase, glycerophosphatase, aminotransferase, ATPase, ADPase, catalase, glucosaminidsae, phosphomonoesterase and NAD nucleosidase (Ramos and Selistre-de-Araujo, 2006).

OPHITOXAEMIA

Ophitoxaemia is the rather exotic term that characterizes the clinical spectrum of snakebite envenomation. Snakebite remains a public health hazard in many regions of the world, particularly in tropical countries (Chippaux, 1998). Even though, it is difficult to be precise about the actual number of cases, it is estimated that the true incidence of snake envenomation could exceed five million per year. About 100,000 of these develop severe sequelae. The global disparity in the epidemiological data reflects variations in health reporting accuracy as well as the diversity of economic and ecological conditions (Chippaux, 1998).

SNAKE ENVENOMATION

Snake envenomation is basically a subcutaneous or intramuscular injection of venom into the prey/human victims. It employs three well integrated strategies. The first and second strategies are prey immobilization, hypotensive and paralysis. This helps in the retardation of the movements and prevents escape from the snake. The third strategy is digestive and degradation of prey tissues assimilation process. The pathophysiology of snake envenomation is a series of complex events that depend on the combined action of toxic and non-toxic components (Warell, 1996), concentration and rate at which toxins diffused from the site of bitten region to the circulation. It includes both local and systemic manifestations.

Ø SYSTEMIC MANIFESTATIONS

The systemic toxicity refers to the distribution of target specific toxins into systemic circulation to get distributed to their sites of action and accounting for the fatality. The systemic manifestations vary with a particular species involved in bite, Elapid venoms produce symptoms as early as 5 minutes (Paul, 1993) or as late as 10 hr (Reid, 1979) after bite, Vipers take slightly longer the mean duration of onset being 20 min. However, symptoms may be delayed for several hr. Sea snake bites almost always

produce myotoxic features within 2 hr so that they are reliably excluded if no symptoms are evident within this period (Paul, 1993). However, the magnitude of systemic toxicity is governed by several factors such as dose of venom injected; rate of diffusion of target specific toxins in to circulation, time gap in between time of bite and time of initiation of anti-venom therapy and also the psychological stress. Following are the popular systemic manifestations.

Neurotoxicity

Neurotoxins are the main toxic components of many snake venoms. They can either inhibit the release of neurotransmitters or bind to the neurotransmitter receptor at postsynaptic site and disrupt synaptic transmission. Thus neurotoxins are divided into two types depending on the mode of action at the neuromuscular junction. Toxins act at the presynaptic site are called the presynaptic neurotoxins and those which act at the post synaptic junction are called the postsynaptic neurotoxins (Yang and Chang, 1999; Liu et al., 2001).

Postsynaptic neurotoxins bind specifically to the nicotinic acetylcholine receptor (nAchR) at the motor end plate and produce a non-depolarizing block of neuromuscular transmission (Lewis and Gutmann, 2004). Over 100 highly homologous postsynaptic neurotoxins have been sequenced. Cobrotoxin, α-bungarotoxin, crotoxin, cerulotoxin, mojave toxin, erabutoxin-b, hostoxin-1, oxylepitoxin-1 and rufoxin are known to exhibit postsynaptic neurotoxicity (Gopalakrishnakone et al., 1980; Bon and Saliou, 1983; Chang, 1985; Tzeng et al., 1986; Bon et al., 1988; Storella et al., 1992; Giron et al., 1996; Nirthanan et al., 2002; French et al., 2004; Beghini et al., 2005; Samson et al., 2005; Tan et al., 2006; Clarke et al., 2006; Lumsden et al., 2007).

Presynaptic action is generally a two-step process. First step is the binding of the toxin the presynaptic site and second step is the action at the presynaptic membrane leading to the blocking of the neurotransmitter release (Schivavo et al., 2000; Rossetto et

al., 2004). Presynaptic neurotoxins have been intensively studied over the last few decades. All known venom presynaptic neurotoxins are PLA_2s as an integral part of their structure (Hawgood and Bon, 1990; Westerlund et al., 1992; Chen et al., 2004). Both monomeric and multimeric β- neurotoxins are found in venoms. Notexin, caudoxin, ammodytoxin (Viljoen et al., 1982; Kiss et al., 2004; Ivanovski et al., 2004) are the very well studied single chain toxins. Taipoxin, textilotoxin, mojave toxin, crotoxin, β-bungarotoxin; clostridial, HDP-1P and HDP-2P are extensively studied multimeric neurotoxins (Wilson et al., 1995; French et al., 2004; Samson et al., 2005; Beghini et al., 2005, Poulsen et al., 2005; Rossetto and Montecucco, 2008; Gao et al., 2009).

Myotoxicity

Myotoxicity is one of the common pharmacological and a serious consequence of snake venom poisoning. Local hemorrhage and necrosis affecting the skin and muscle layers are the chief manifestations of myotoxicity. Myotoxicity may be due to the vascular degeneration and ischemia caused by venom metalloproteinases or it may result from a direct action of myotoxins (enzymatic/nonenzymatic) on the plasma membrane of muscle cells, which is evident from the rapid release of cytoplasmic markers, creatine kinase (CK) and lactate dehydrogenase (LDH) accompanied by the prominent increase in total muscle calcium ion (Rucavado and Lomonte, 1996; Gutierrez and Lomonte, 1997; Salvini et al., 2001). The increased influx of calcium ion leads to the cell death. Myotoxicity is associated with many presynaptically acting neurotoxins (Gopalakrishnakone et al., 1980; Ziolkowske and Bieber, 1992). In addition, several myonecrotic polypeptides and myotoxic PLA_2 enzymes have been isolated and characterized from various snake venoms (Mebs, 1986; Mebs and Samejima, 1986; Kasturi and Gowda, 1989; Lomonte et al., 1994; Radis-Baptista et al., 1999; Rodrigues et al., 2007; Murakami et al., 2008; de Freitas Oliveira et al., 2009).

Cardiotoxicity

Circulator disturbance is one of the major effects during snake envenomation. This could be due to the formation of internal clots by the venom components or binding at a specific target site on cardiac muscle cells and increasing the intracellular calcium ion concentration (Huang et al., 1993).

Cardiotoxins are small molecular mass (5.5–7 kDa) highly basic proteins and contain about 60 amino acid residues and cross-linked by four disulfide bridges (Jang et al., 1997) and belongs to three finger toxins family (Dufton and Hider, 1983). These cardiotoxins are associated with many pharmacological properties such as hemolysis, contraction of cardiac muscle and inhibition of protein kinase C (Mirtechin, 1991; Cher., et al., 2005). Hemolytic and cytolytic properties of cardiotoxins are due to asymmetric distribution of hydrophobic and hydrophilic amino acids (Kini and Evans, 1989). Cardiotoxin I, II, III and IV (Bhaskaran et al., 1994), and cardiotoxin-like basic protein from *Naja atra* venom (Rong et al., 2007), sagitoxin from *Naja saggitifera* (Mir et al., 2008) and a few PLA_2 enzymes from the venoms of *Naja nigricollis* (Fletcher et al., 1982), *Bungarus fasciatus* (Chang et al., 1983), *Ophiophagus hannah* (Huang et al., 1993; Wang et al., 2001), and *Naja naja* (Cher et al., 2005) venoms were reported to exhibit cardiotoxicity.

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Cytotoxicity

Snake venom PLA_2 enzymes are known to contain cytotoxic activity (Dufton and Hider, 1983; Fletcher and Jiang, 1993). Cytotoxic PLA_2s have been isolated and studied from the venom of *Notechis scutellatus scutellatus* (Ho et al., 1986), *Naja nigricollis* (Chwetzoff et al., 1989), *Naja naja* (Basavarajappa and Gowda, 1992; Rudrammaji, 1994), *Naja nigricollis* (Gowda and Middlebrook, 1993), *Oxyuranus scutellatus scutellatus* (Poulsen et al., 2005) and *Daboia russellii* (Choudhury et al., 2006; Maity et al., 2007; Gomes et al., 2007). A cytotoxin-like protein purified from the venom of *Ophiophagus hannah* (Chang et al., 2006).

Hemolytic activity

Hemolysis is the disruption of the red blood cells resulting in the release of hemoglobin and other cellular contents to the medium. Based on their mode of action, snake venoms have been classified into two major groups; the direct lytic venoms which are capable of hemolyzing washed red blood cells through hydrolysis of phospholipids at different domains of erythrocytes and indirect hemolysis has been shown to be mediated by lysophosphatides produced through the hydrolytic action of the venom phospholiase A_2. PLA_2s causing direct hemolysis have been reported from *Trimeresurus flavoviridis* (Vishwanath and Gowda, 1987), *Naja naja* (Bhat and Gowda, 1989; Basavarajappa and Gowda, 1992), *Daboia russsellii* (Vishwanath et al., 1987; Kasturi and Gowda, 1989, 1992; Chakrabarty et al., 2002; Tsai et al., 2007; Maity et al., 2007). The indirect hemolysis has been shown to be mediated by lysophospholipids and hemolytic agents (Welzein, 1979). All PLA_2s shows indirect hemolytic activity. The non-hemolytic phospholipases hydrolyze only the externally oriented phospholipids in the erythrocyte membrane (Rosenberg et al., 1983). The relationship between hemolytic activity and phospholipid hydrolysis in erythrocytes has been reviewed by Condrea et al., (1979).

Coagulant activity (pro/anticoagulant)

Blood coagulation is the result of a series of zymogen activation. Over 30 different substances that affect blood coagulation have been found in the blood and tissues. Some promoting the coagulation are called pro-coagulants and others inhibiting the coagulation are called anti-coagulants, whether or not, the blood will coagulate depends on the degree of balance between these two groups of substances (Kini, 2005). Normally, the anticoagulants predominate and thus the blood does not coagulate, but at the site of trauma the activity of pro-coagulants becomes much greater than that of anti-coagulants and then clot does develop. Snake venoms contain many biologically active proteins, which effect different steps in blood coagulation (Teng et al., 1985). Snake venom proteins such as PLA_2s, proteases such as thrombin-like enzymes, prothrombin

activators, factor V activators, factor X activators, fibrin(ogen)olytic and factor X binding proteins and non-enzymatic polypepetides are known to interfere in blood coagulation process (Markland, 1998; Huang et al., 1999; Matsui et al., 2000; Samel et al., 2002; Satish et al., 2004).

Platelet aggregation (activation/inhibition)

Platelet aggregation means platelets sticking to other platelets rather than to a different surface. In the circulating blood, discoidal platelets are considered to be in the resting state and in this state; they do not readily adhere to any surface and circulate freely to survey the integrity of inner lining of blood vessels. They become sticky when there is any damage to the vascular endothelium and aggregate at sites of newly exposed proteins such as collagen and von Willebrand factor (vWF). Platelets aggregate into a mass at the site of vascular injury and forms hemostatic plug, which seals off the break in the blood vessel.

Platelets become sticky upon stimulation by diverse agonists which includes small molecular weight compounds such as ADP, arachidonate, serotonin and epinephrine; enzymes such as thrombin and trypsin; particulate materials, such as collagen and antigen–antibody complexes; lipids, such as platelet activating factor (PAF) and ionophores, such as A23187 (Zucker, 1989). Stimulation by these diverse agonists initiates a series of cellular responses such as adhesion, change in platelets shape from disc to sphere and release of various substances (Kini and Evans, 1990). In general venom toxins (enzymatic/non-enzymatic) may promote or inhibit agonists induced aggregation of platelets. Enzymatic and non-enzymatic platelet aggregating factors have been isolated from many different snake venoms (Kini and Evans, 1990; Ouyang et al., 1992; Kamiguti et al., 1998; Kemparaju et al., 1999; Siigur and Siigur, 2000; Jagadeesha et al., 2002; Watanabe et al., 2003; Satish et al., 2004; Wang et al., 2005; Serrano et al., 2007; Marcussi et al., 2007; Sanchez et al., 2009).

Hypotensive activity

Most snake venoms employ a variety of means to induce rapid and profound hypotension, leading to circulatory shock, prey immobilization and death. Snake venoms are known to induce hypotension accompanied by decrease in pulmonary pressure due to the release of pharmacologically active autocoids such as histamine, 5–hydroxy tryptamine and leukotrienes (Rothschild and Rothschild, 1979). Many crotaline venoms posses' hypotensive peptides of 5-13 amino acids that are N-terminally blocked with pyroglutamic acid. These peptides are generally known as bradykinin-potentiating peptides (BPPs) because of their capacity to enhance the hypotensive effects of bradykinin. An acidic PLA_2 from *Daboia russsellii* venom showed hypotensive activity accompanied by decrease in pulmonary pressure (Huang and Lee, 1984). Indomethacin, a known PLA_2 inhibitor, significantly reduces the hypotensive action of various snake venom phospholipases (Ho and Lee, 1981). This suggests that the synthesis and subsequent release of prostaglandins appears to be very critical in the PLA_2 induced hypotension.

Convulsant activity

Death following cobra envenomation is often preceded by convulsion due to asphyxia arising from respiratory paralysis and other pre-agonial effects. Snake venom components known to cause depletion of stored acetylcholine due to high influx of potassium ions. The nerve does not release the neurotransmitter (Karlsson, 1979) there by these acts as presynaptic neurotoxins. The convulsant activity has been reported from the venoms of *Daboia russsellii* (Kasturi and Gowda, 1989; Jayanthi and Gowda, 1990), *Naja naja* (Bhat and Gowda, 1991) and *Echis carinatus* (Kemparaju et al., 1994).

Ø LOCAL MANIFESTATIONS

Local changes are the earliest manifestations of snake bite. Features are noted within 6-8 minutes but may have onset up to 30 min (Reid and Theakston, 1983). Development of a small reddish wheal is the first to occur. This is followed by swelling resulting in edema, echymoses (pinpoint red spots on the skin) and hemorrhage are usually apparent within minutes of the bite. Viperid venoms are known to cause local effects, which frequently include pain, swelling, echymoses and local hemorrhage. Such signs are sometimes followed by liquifaction of the area surrounding the bite. The local area of the bite may become devascularized with features of necrosis predisposing to onset of gangrenous changes. Secondary infection including formation of an erythematous area inside of which is a pale ischemic region that develops a dark necrotic center as the lesion matures. Healing is slow and these ulcers may persist for months leaving a deep scar. Following are the local effects.

Edema forming activity

A very common and early local effect of snake bite is swelling and edema of the bitten region. Edema formerly known as dropsy or hydropsy is the increase of interstitial fluid in any tissue or organ. Generally, the amount of interstitial fluid is in the balance of homeostasis. Increased secretion of fluid into the interstitium or impaired removal of this fluid may cause edema. The action on the vessels is brought about by either the direct action of venom toxins affecting the microvasculature (Chaves et al., 1995) or more commonly by the formation of autocoids and other vasoactive compounds by the PLA_2 action of the toxins. The edema induced by *Bothrops jararaca* venom is mediated by cyclooxygenase and lipoxygenase eicosanoid products and by the action of L_1 and L_2 adrenergic receptors (Trebien and Calixto, 1989). Pretreatment with indomethacin, a well-known inhibitor of the cyclooxygenase pathway reduced the edema induced by *Bothrops asper* and *Bothrops jararaca* venoms. It is suggested that PLA_2 induce by two different mechanisms (a) by releasing arachidonic acid as a membrane phospholipids,

leading to the biosynthesis of eicosanoids and (b) by directly affecting the microvasculature, there by causing plasma exudation (Chaves et al., 1995).

PLA_2s are cytotoxic to mast cells and cause their degranulation and releases physiological mediators like histamine, serotonin, leukotriens, which increases vascular permeability (Kasturi and Gowda, 1992). The venoms of *Trimeresurus flavoviridis* (Vishwanath et al., 1987; Yamaguchi et al., 2001), *Trimeresurus mucrosquamatus* (Teng et al., 1989; Chiu et al., 1989), *Daboia russsellii* (Kasturi and Gowda, 1989), *Naja naja* (Bhat and Gowda, 1989; Basavarajappa and Gowda, 1992), *Echis carinatus* (Kemparaju et al., 1994), *Bothrops asper* (Lomonte et al., 1993; Chaves et al., 1995) and *Bothrops lanceolatus* (de Faria et al., 2001) are reported to induce edema.

Hemorrhagic activity

Hemorrhage is the medical term for bleeding. In common usage, a hemorrhage means particularly severe bleeding; although technically it means escape of blood to extravascular space, with the damage to the microvessel blood wall. “Hemorrhagins” the term was introduced by Grotto et al (1967). The main factor responsible for hemorrhage is zinc dependent metalloproteases of ‘metzincin’ family enzymes which comprise a major group of active principles in Viper venom. The enzymatic degradation of the vascular basement membrane underlying endothelial cells of capillaries is assumed to be a major cause of hemorrhage. Damaged vascular basement membranes and rupture of the endothelial cell membranes in the hemorrhagic area have been observed in histological studies (Ownby et al., 1990; Kamiguti et al., 1991, 1996; Gutierrez et al., 2005, 2009).

Hemorrhagic activity has been associated with proteolytic activity. Treatment with EDTA abolishes both proteolytic and hemorrhagic effects (Bjarnason and Fox, 1988, 1995). Of the 65 hemorrhagic toxins, 12 have been analyzed for their metal content, all of them have been found to contain zinc and many more are inhibited by metal chealtors. Ten of the twelve toxins contained approximately 1 mole of zinc per

mole of toxin (Bjarnason and Fox, 1994). Therefore, the venom induced hemorrhage is primarily caused by metal dependent proteolytic activities of the hemorrhagic toxins, probably acting on connective tissue and basement membrane components.

There appear to be three possible mechanisms involved in basement membrane degradation; (1) that caused by direct proteolytic effects of hemorrhagic factors; (2) that caused by secondary activated proteins after envenomation and (3) a combination of the above two mechanisms.

Although many hemorrhagic toxins have been well characterized biochemically, relatively little is known about the pathogenesis of hemorrhage induced by these toxins. There are two possible ways in which hemorrhage can occur (Ownby, 1982). In hemorrhage "per diapedesis", the intercellular junctions between capillary endothelial cells widen allowing blood to escape through them into the connective tissue space. HR-1, HR -2a and HR -2b from *Trimeresurus flavoviridis* (Ohasaka, 1979) and BaH1 from *Bothrops asper* (Borkow et al., 1995) venoms were found to exhibit "per diapedesis" mechanism. In hemorrhage "per rhexis", the capillary endothelial cells undergo lysis leading to large intracellular gaps, i,e within the endothelial cells, through which blood escapes. Some of the enzymes studied are as fallowes HT-1 and HT-2 from *Critakys rubber rubber* (Obrig et al., 1993), Atrolysin a from *Crotalus atrox* (Obrig et al., 1993) and Proteinase IV from *Crotalus adamenteus* (Anderson and Ownby, 1977) venoms.

In addition, some hemorrhagins also possess other biological activities, for example, myonecrotic (Bilitoxin and baH1), fibrinogenolytic (Atrolysin f and Jararhagin), platelet aggregation inhibition (Atrolysin a) (Gutierrez and Rucavado, 2000; Gutierrez et al., 2009 and references there in) properties. Many hemorrhagic toxins have been purified and characterized biochemically from the venoms of *Crotalus atrox* (Bjarnason and Fox, 1987; Baramova et al., 1989), *Agkistrodon bilineatus bilineatus* (Imai et al., 1989), *Agkistrodon halys blomhoffii* (Ownby et al., 1990), *Bothrops jararaca* (Anai et al., 2002; Assakura et al., 2003), *Bothrops asper* (Gutierrez et al., 1995;

Franceschi et al., 2000), *Bothrops atrox* (Petretski et al., 2000), *Bothrops lanceolatus* (Stroka et al., 2005), *Bothrops lanceolatus* (Neto and Marques, 2005), *Bothrops jararacussu* (Mazzi et al., 2004), *Bothrops neuwiedi* (Mandelbaum et al., 1982), *Bothrops moojeni* (Gomes et al., 2009), *Dabioa russsellii* (Chakrabarty et al., 1993, 2000; Kole et al., 2000; Mukherjee, 2008; Chen, et al., 2008).

Dermonecrosis / Myonecrosis

Necrosis at the bitten region is the main characteristic features of the Viperid snake envenomation. Necrosis is due to direct action of myotoxins (enzymatic/non-enzymatic) or hemorrhagic metalloproteinases on skin and muscle cells causes extensive skin and muscle damage resulting in weakness of muscle and painful restriction of movements with muscle tenderness. Massive destruction of muscle leads to release of muscle enzymes and proteins, as evidenced by a rise in serum CK and LDH activities. If muscle damage is severe, recovery is delayed, with wasting of the muscles involved (Gopalkrishnakone et al., 1997; Franceschi et al., 2000; Leon et al., 2000; Gutierrez et al., 2009 and references there in).

POSSIBLE AGENTS OF LOCAL TOXICITY

The induction of systemic effects depends upon the concentration, rate at which toxins diffused into the systemic circulation from the site of bitten region to the target site. Before exerting the systemic effects, extensive local tissue destruction would take place by degrading the components of extracellular matrix (ECM) and connective tissue surrounding the blood vessels and capillaries. Hemorrhagic metalloproteinases [homologous to matrix metalloproteinases (MMPs)] and hyaluronidases (glycosidases) are considered to be the primary agents responsible. In addition, locally acting myotoxins (enzymatic/non-enzymatic) also participate in local tissue damage (Yingprasertchai et al., 2003; Gutierrez et al., 2009).

EXTRACELLULAR MATRIX (ECM)

Extracellular matrix (ECM) is a diverse, complex structural entity of animal system. Many of the cells in tissues of multi-cellular organisms are embedded in an ECM consisting of secreted proteins and polysaccharides. The structure and function greatly vary with the nature of the tissue with which it is associated, viz. bone, and cartilage, connective tissue surrounding glands and blood vessels, and the intercellular cementing scaffold. ECM consists of three classes of molecules.

Structural proteins: The major structural protein of the ECM is collagens and elastins, their percentage of abundance appear to vary depending upon the nature of the tissue. For example, collagen is the single most abundant protein in skin and cartilaginous tissues while, elastin is abundant in basement membrane and other contractile tissues that regularly stretch and then return to this original shape. Further, collagens vary in their relative abundance, for example, collagen type I is abundant in skin while collagen type IV is abundant in basement membrane. Collagens form a network with laminin viz. nidogen/entactin with other protein and proteoglycans to form a stable basement membrane and offer mechanical support.

Glycosaminoglycans: Gylcosaminoglycans (GAGs) are the most abundant heteropolysaccharides in the body, which are long unbranched polysaccharides containing a repeating disaccharide unit. These include chondroitin, chondroitin sulfate, dermatan sulfate, keratan sulfate and hyaluronic acid. GAGs, because of their negative charge can hold large amount of cations and as well as water, thus serve as a reservoir of extracellular cations and as well as water. All glycosaminoglycans (GAGs), except hyaluronic acid are sulfated and bound covalently to any of the core proteins and forms proteoglycan complexes. Hyaluronan is a long linear thread like polymer; it holds aggrecan monomers and structural proteins non-covalently throughout its length.

Adhesive glycoproteins: These include fibronectin, laminin and nidogen/entactin. Fibronectin is the principle adhesive glycoprotein of connective tissues, it has binding site for both collagen and GAGs hence, it crosslink the matrix components.

A distinct site on the fibronectin molecule is recognized by cell surface receptors and these responsible for the attachment of the cells to the ECM. Laminin is more abundant in basement membrane and it can self assemble into mesh like polymers; such net works are the major structural components of the basal lamina. Laminin also has binding sites for all cell surface receptors and collagen type IV. In addition, laminins are highly associated with another adhesive protein entactin/nidogen which also binds to collagen type IV. As a result of these multiple interactions, adhesive glycoproteins act as cementing materials and bridge structural proteins and the integrins of cells through their RGD sequence.

ECM play role in several vital functions such as embryo development, migration, adhesion, proliferation, differentiation and maintenance of epithelial morphology, immune-surveillance, inflammation, wound healing, angiogenesis, malignant transformation and visco-elastic functions (Girish et al., 2009 and references therein). The remodeling of the ECM and the dynamic state of the matrix molecules must, therefore play a critical role in both normal and pathological processes.

EXTRACELLULAR MATRIX DEGRADING ENZYMES

In snake venoms, two groups of enzymes degrade the components of extracellular matrix, namely, hyaluronidases, which primarily degrade the hyaluronic acid and the metalloproteinases which are popularly known as (SVMPs) and degrade protein scaffold.

Ø *HYALURONIDASES*

Hyaluronidases are endo-β-glycosidases and are distributed widely in nature (Girish and Kemparaju, 2007). They are found in various human organs and body fluids, and in external secretions of viruses, bacteriophages, bacteria (Stern and Jedrzejas, 2006), fungi (Shimizu et al., 1995), nematode, leeches (Frost et al., 1996) and in the venom of snakes (Xu et al., 1982; Kudo and Tu, 2001; Girish et al., 2004(b); Girish and Kemparaju, 2005), scorpions (Ramanaiah et al., 1990; Pessini et al., 2001), bees (Gmachl and Kreil, 1993; Markovic-Housley et al., 2000), wasps (Kreil, 1995), hornets (Lu et al.,

1995), spiders (Rash and Hodgson, 2002; Nagaraju et al., 2006), caterpillars (da C B Gouveia et al., 2005), fishes (Poh et al., 1992; Ng et al., 2005) and lizards (Tu and Hendon, 1983). Hyaluronidases have been classified into three types based on their mechanism of action and end product analysis (Meyer, 1971).

1) Hyaluronate 4-glycanohydrolases/hyaluronoglucosaminidases/ endo β-N-acetylhexosaminidases (EC 3.2.1.35) that hydrolyze β 1-4 glycosidic bond yielding tetrasaccharides and hexasaccharides as major end products with N-acetyl glucosamine and glucuronic acid placed respectively in reducing and non-reducing end of the products, e.g. hyaluronidases from lysosome, spermatozoa, snake venom, etc.

2) Hyaluronate 3-glycanohydrolases/hyaloglucuronidases/endo β-glucuronidases (EC 3.2.1.36) that hydrolyze β 1-3 glycosidic bond yielding tetrasaccharide and hexasaccharides (GlcNAc-GlcUA-GlcNAc-GlcUA) as major end-products with glucuronic acid and N-acetyl glucosamine are placed respectively in reducing and non-reducing end of the products, e.g. hyaluronidase from salivary glands of leeches, crustaceans, etc.

3) Hyaluronate lyases/endo β-N-acetylhexosaminidases (EC 4.2.99.1) that hydrolyze β 1-4 glycosidic bond yielding predominantly disaccharides as end products with N-acetyl glucosamine and glucuronic acid placed respectively in reducing and non-reducing ends of the products e.g. microbial hyaluronidases.

The earlier two types are hydrolases as the mechanism involves hydrolytic cleavage while the last type is an eliminase/lyase due to its β-elimination with the introduction of a double bond yielding 4, 5 unsaturated glucuronic acid containing disaccharides. All hyaluronidases predominantly degrade hyaluronan however, also cleaves other glycosaminoglycans (GAGs) such as chondroitin and chondroitin sulfates to a lesser extent (Baker et al., 2002; Stern and Jedrzejas, 2006). Strikingly, at pH 7.0, bovine testicular hyaluronidase catalyzes the formation of glycosidic bond through transglycosylation reaction, while at pH 5.0 it will cleave the glycosidic bond by its glycosidase activity (Saitoh et al., 1995). In addition to the above classification, based on

pH activity profile, hyaluronidases are loosely classified in to two types; acidic active enzymes, active between pH 3 and 4 e.g. human liver and serum hyaluronidases and, neutral active enzymes, active between pH 5 and 8 e.g. ovine testicular, snake and other venom hyaluronidases (Frost et al., 1996; Girish and Kemparaju, 2006).

The substrate of hyaluronidases, the hyaluronan is a high molecular weight, non-sulfated, linear, acidic GAG and found throughout the animal kingdom, especially in the ECM of soft connective tissues that connects protein filaments, collagen fibers and the connective tissue cells (Laurent, 1989; Laurent and Fraser, 1992). It is composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked through (β1, 3 and 1, 4) glycosidic linkages. In contrast to other GAGs, hyaluronan is not in covalent bridge with any of ECM proteins.

Hyaluronidases and hyaluronan system is implicated in such diverse biological functions as embryogenesis, cell migration, wound healing, tissue turn over and malignancies (Laurent and Fraser, 1992; Spicer and McDonald, 1998; Lee and Spicer, 2000; Girish and Kemparaju, 2007).

In venoms, hyaluronidase is popularly referred to as a 'spreading factor' as it facilitates easy diffusion of systemic toxins in to the circulation of the prey. Rapid hydrolysis of long linear megastructure of hyaluronan into fragments of varied molecular size drastically reduces the viscosity of the envenomed milieu favoring rapid diffusion of toxins into circulation which were otherwise diffused much slowly (Girish et al., 2002, 2004(b); Girish and Kemparaju, 2006).

Structure and mechanism of action

Despite a long history, on snake venom hyaluronidases no information so far available on the active site residues and mechanism of hyaluronan degradation. The structural elucidation of snake venom hyaluronidase was an untouched area till recently. However, Harrison et al., (2007) deduced the full length sequence of *Echis ocellatus* hyaluronidase based on the venom gland cDNA sequences. This was the first ever

sequence deduced for snake venom hyaluronidase enzyme. The enzyme has five conserved N-linked glycosylation sites, three of which appeared to be highly potential for glycosylation. Therefore, the final product appeared to be glycosylated with the predicted mass of well over 50 kDa. This hyaluronidase is in great homology, over 95% with *Echis pyramidum leakeyi*, *Bitis arietans* and *Cerastes cerastes cerastes* enzymes. They have conserved catalytic (Glu^{135}), positional (Asp^{133}, Tyr^{206}, Tyr^{253} and Trp^{328}) and cysteine scaffold (Cys^{340}, Cys^{211}, Cys^{227}, Cys^{365}, Cys^{370}, Cys^{376} and Cys^{429}). In contrast, the enzyme share sequence identity to the extent of 31% to 40% with the honey bee (*Apis mellifera*), wasps (*Vespula vulgaris*), stonefish (*Synanceja horrida*) and puffer fish (*Tetraodon nigroviridis*) venom enzymes but much higher for mammalian enzymes. However, it showed highest identity of 50% to dog (*Canis familiaris*) hyaluronidase 4. In the recent past stonefish (*Synanceja horrida*) hyaluronidase (SFHYA1) sequence was deduced from cDNA and expressed in an active form. This was the first aquatic enzyme to deduce its structure (Ng et al., 2005).

The only hydrolase class of hyaluronidase has an established 3D structure determined through X-ray crystallography was from bee (*Apis mellifera*) venom. The structure was established in its native form and also in complex with the hyaluronan oligomer. It is a globular single-domain protein with approximate core dimensions of 52 x 44 x 39 A^o. It has ten α helices, eleven β strands and six 3_{10} helices. It contains four potential glycosylation sites and two disulfide bridges stabilizing the structure. The overall topology resembles a classical eight stranded, $(\beta/\alpha)_8$ Triose phosphate isomerase (TIM) barrel motif except that the barrel contains of only seven strands (Markovic-Housley et al., 2000).

Interestingly, when superimposed viper venom hyaluronidase sequence on to the crystal structure of *Apis mellifera* (Fig. 1.1), conservation of 3D structural scaffold observed (Harrison et al., 2007). The regular $(\beta/\alpha)_8$ TIM barrel motif has been found in most of the well-known glycosidases including, α-amylase, β-amylase and β-glucuronidase enzymes (Rigden et al., 2003).

The precise mechanism of hyaluronan degradation by vertebrate hyaluronidase although not clear, the crystal structure of bee venom enzyme which is a vertebrate-like enzyme provides an insight into the mode of substrate binding and the catalytic site of the enzyme (Markovic-Housley et al., 2000). The enzyme has a substrate-binding cleft. The cleft is large enough to bind the hexasaccharide unit of the polymer. The substrate binding appears to induce an allosteric modification of the enzyme secondary structures. The mechanism appears to be acid/base-catalyzed reaction, in which a double displacement event at anomeric carbon atom of N-acetyl-D-glucosamine of the polymer has been proposed. Such a process appears not to be involved in covalent intermediate (enzyme-substrate complex) formation. Albeit, an oxocarbonium ion-like transition state, and retention of β-configuration of C1 of N-acetyl-D-glucosamine of the product seen. Two carboxylic acid/acidic residues form the catalytic site, one donating the proton and the other acting as the nucleophile. Asp 111 and Glu 113 are the strictly conserved acidic residues in the hyaluronan-binding groove of the enzyme. Glu 113 appear to act as proton donor as its side chain carboxylic group positioned with a distance of 2.6 A^o close to the glycosidic oxygen, while, the side chain of Asp 111 is placed more than 5 A^o. Indeed, for steric reasons, Glu 113 and Asp 111 side chains cannot approach the anomeric carbon atom of the susceptible glycosidic bond of the substrate from opposite sides. Hence, substrate-assisted reaction has been considered, N-acetyl carbonyl group at C2 of the N-acetyl-D-glucosamine of the substrate appears to act as a nucleophilic base in which, the accurate positioning of N-acetyl side chain with respect to the enzyme is a prerequisite. Reaction terminates by water splitting and incorporation of OH^- into C1 of N-acetyl-D-glucosamine and H^+ into Glu 113. A 3D model of bovine PH-20 has also been deduced; effort in understanding the mechanism of glycosidic bond cleavage and the functional analysis of mutants indicated the importance of Glu113 over Asp111. Substitution of Gln for Glu 113 and Asn for Asp111 in pH-20 resulted in no detectable activity in the earlier case, while about 3% residual activity observed in the later case (Arming et al., 1997). As the active site residues of mammalian and venom hyaluronidases are conserved and also

the retention of the 3D scaffold, a common catalytic mechanism has been proposed (Fig. 1.2).

Based on the 3D structure of bee venom hyaluronidase, the structures of human hyaluronidases are modeled. The comparison between the simulated models of human hyaluronidases and the experimental structure of bee venom enzyme suggests that all hyaluronidases have 3D structures that are similar to one another and also similar to that of bee venom enzyme. Therefore, presumably these models are similar to all other vertebrate enzymes. Based on high homology in primary, secondary, and tertiary structures of bee venom HAase and and site directed mutagenesis of human PH-20 enzyme, it is proposed that degradation by human enzymes may proceed *via* a doubledisplacement mechanism, with retention of the HA substrate conformation (Jedrzejas and Stern, 2005; Stern and Jedrzejas, 2006).

Among microbial HAases, the enzymes from *S. pneumoniae* (S. PHL) and *P. agalactiae* are well characterized. The 3D X-ray analysis indicates that the enzyme is a globular protein with two distinct structural domains connected by a short peptide linker. The N-terminal α-helical domain contains the first 361 amino acid residues of the active form of the enzyme. The C-terminal β-sheet domain contains 347 residues, and Asn 349, His 399 and Tyr 408 are identified as catalytic residues in the functional enzyme. Moreover, a recent X-ray crystallography study on the structure of *S. agalactiae* HA lyase has revealed the similar structure of S. PHL in the architecture of the entire enzyme as well as in the active site geometry (Jedrzejas, 2000, 2001; Stern and Jedrzejas, 2006). The active center of these HA lyases is composed of two main parts, a catalytic group responsible for substrate degradation and an aromatic patch responsible for the selection of cleavage sites on the substrate chains (Jedrzejas, 2000). The HA lyases from *S. pneumoniae, S. agalactiae* and *S. aures* showed a high degree of similarity, with global similarities above 65% and local similarities of about 80% (Jedrzejas, 2000; Stern and Jedrzejas, 2006). Through X-ray crystallography studies including (a) native enzyme, (b) complexes between the enzyme and degradation products such as disaccharides,

tetrasaccharides and hexasaccharides, and (c) site-directed mutagenesis (Jedrzejas, 2000) proposed a detailed catalytic mechanism called the proton acceptance and donation (PDA) mechanism (Stern and Jedrzejas, 2006). This mechanism involves an acceptance of proton by the enzyme followed by the donation of a different proton from the enzyme to the β 1-4 glycosidic oxygen. The degradation process consists of five distinctive steps (Jedrzejas, 2000; Stern and Jedrzejas, 2006).

Ø *SNAKE VENOM METALLOPROTEINASES (SVMPs)*

SVMPs are the principal destructive agents especially in Viper venoms. These are largely Zn^{+2} containing metalloproteases belongs to metzincin family. These are primarily responsible for the venom induced hemorrhage and this is due to the degradation of proteins in basement membrane of blood vessels (Gutierrez and Rucavado, 2000; Fox and Serrano, 2005; Gutierrez et al., 2009). This leads to the collapse in the integrity of capillary walls resulting in hemorrhage at the envenomed region (Moura-da-silva et al., 2003). In addition, local edema, necrosis and blistering are also seen. The extensive tissue degradation might result in poor tissue regeneration. However, these proteases might also act systemically giving rise to serious complications such as alterations of hemostatic mechanisms (Kamiguti et al., 1996; Warrel, 1996; Markland, 1998; Gutierrez et al., 2005, 2009 and references there in), impairment of coagulation mechanisms or alterations in platelet counts or function resulting in local and systemic bleeding, cardiovascular shock, pulmanory bleeding and hemorrhage in the central nervous system (Fig. 1.3). There are reports of over 100 SVMPs from 36 species of snakes predominantly of Viperid family (Bjarnason and Fox, 1995 and references are there in). The sequence of 20 SVMPs are now known (Bjarnason and Fox, 1994; Hite et al., 1994). Crystal structures of several SVMPs have been described from various snake venoms they are, adamalysin II from *Crotalus adamanteus* (Gomis-Ruth, et al., 1993), atrolysin C from *Crotalus atrox* (Zhang et al., 1994), H2-proteinase from *Trimeresurus flavoviridis* (Kumasaka et al., 1996), acutolysins A and C from *Agkistrodon acutus* (Gong

et al., 1998; Zhu et al., 1999), Jararhagin and Bothropasin from *Bothrops jararaca* (de Souza et al., 2001; Muniz et al., 2008), TM-3 from *Trimeresurus mucrosquamatus* (Huang et al., 2002), Vascular apoptosis-inducing protein (VAP-1 and 2) from *Crotalus atrox* (Igarashi et al., 2006, 2007), BaP1 from *Bothropus asper* (Watanabe et al., 2003; Lingott et al., 2009), Factor X activator (RVV-X) from *Daboia russellii* venom (Takeda et al., 2007).

Based on molecular masses and their domain structure, SVMPs have been categorized into four classes; P-1 to P-IV (Fig. 1.4). Class P-I, includes enzymes with molecular mass of 20-30 kDa and contains only metalloproteinase domain; Class P-II, includes enzymes with molecular mass of 30-50 kDa and contains metalloproteinase domain and disintegrin like domain (Dis-like domain); Class P-III, includes enzymes with molecular mass of 50-80 kDa and contains metalloproteinase domain, disintegrin like domain and cysteine-Dis-like domains; Class P-IV, includes enzymes with molecular mass of 80-100 kDa and contain lectin binding domain(s) in addition to disintegrin-like domain and cysteine rich domain (Fox and Serrano, 2005).

Domains in snake venom metalloproteases (SVMPs)

Protease domain

The primary functional characteristics of snake venom metalloproteases reside in the protease domain. This domain ranges from 202 to 204 amino acid residues in length. Based on the number of disulfide bonds present, the protease domains are of two types, two disulfide type and three disulfide type. Three disulfide type protease domain is characteristic of class-II, III and class IV enzymes, while class I enzymes posses either of the protease domains.

The X-ray crystallography structures of adamalysin (Gomis – Ruth et al., 1993) and atrolysin C (Zhang et al., 1994) have provided insight and helped in understanding the structural characteristics of this domain. The domain assumes an oblate ellipsoidal

shape. The active site cleft has a zinc ion tetrahedrally coordinated to three histidine residues, His, 142, His 146 and His 152 and to a water molecule. In addition, Glu 143 is also found in the active site. The active site cleft divides the protease domain into an upper major domain with a twisted five stranded β sheet surrounded by four α- helices, and a lower smaller domain with many turns ending in an α-helix.

Disintegrin / disintegrin - like domains

Disintegrins are a family of proteins isolated from snake venoms. These are non–enzymatic, low molecular weight polypeptides which contain RGD sequence (Arg-Glu-Asp). They range in size from 49 to 84 amino acid residues in length and have been found in many Viperid and Crotalid venoms. Trigramin was the first disintegrin characterized from the venom of *Trimeresurus gramineus*. Following this, a number of disintegrins have been reported and reviewed from snake venoms. Disintegrin–like domains have atleast one additional cysteinyl residue in the structure and devoid of RGD sequence (Jia et al., 1996). The proteins containing disintegrin–like domain have been studied from the venoms of *Crotalus atrox* (hemorrhagic toxin a and e), *Daboia russelli* (RVVX) (Hite et al., 1994 and references therein). Large metalloproteases composed of N-terminal metalloprotease domain, a disintegrin–like domain and C-terminal cysteine rich terminus (MDC). Metalloproteases containing disintegrin / disintegrin-like domain from various snake venoms are known to cause local hemorrhage and systemic bleeding by consuming clotting factors in the envenomated victims and they are the potent inhibitors of platelet aggregation process. (Hite et al., 1994; Jia et al., 1996). P-I class of metalloproteases are devoid of disintegrin domain. In P-II class although this domain is present at the mRNA level, during maturation, disintegrin domain is removed either by autolytic event or by the action of other proteases. The P-III and P-IV classes' posse's only the disintegrin–like domain.

Spacer region

The spacer region is a stretch of 21 amino acid residues that follows the protease domain. In the P-I class this region is processed from the matured protease domain. While in P-II to P-IV proteins this region seperates the protease domain from the disintegrin-(like) domain (Shannon et al., 1989). The cysteine residue is normally found within the spacer region and is assumed to be involved in a disulfide bonding with an unpaired cysteinyl residue which is present in the disintegrin–like domain. Thus it may stabilize the multidomain structure (Fox and Bjaranason, 1995).

Cysteine – rich domain

This domain is characterized by the presence of high content of cysteine residues. The exact function of this domain is unknown. Despite, one can speculate that it plays an important role in the molecular presentation of the disintegrin–like domain and probably it is involved in the protein–protein interactions and also participate in intradomain disulfide bonding and thus stabilizing the multi-domain structure (Jia et al., 1996). From the cDNA sequences of Atrolysin E and haemorrhagic toxins from *Crotalus atrox* venom suggests that SVMPs are synthesized as pre-, pro-, proteases. Highly conserved 18-residues stretch of amino acids which serve as a signal sequence in Pre-domain. Pro domain consists of a conserved sequence PRCGVPD, called a cysteine switch which is responsible for the enzyme activation and is lacking in the matured protein. Comparison of amino acid sequences of a matured protein indicated that, despite differences in their molecular size, the snake venom metalloproteases may be related through a common ancestral gene encoding prodomain, metalloprotease, disintegrin and cysteine rich domains. These metalloproteases are sensitive to the chelators such as EDTA, EGTA, cyanide and 1, 10 phenanthroline.

SNAKE BITE TREATMENT

Several folk cures are practiced even to this day in rural India such as split chicken treatment, snake stone application, ligation, amputation, botanical cure etc. Except for botanical cure, remaining offered less or no protection. The botanical cure is highly impressive and most popularly used in all folklore remedies for snakebite. The application of formulations prepared from various plants parts was applied topically to the bitten region or given orally as liquid extract is still in practice. Many plant extracts and their isolates have proven the anti-venom activity (Mors et al., 2000; Soares et al., 2005 and references there in) (Table. 1.2). In India the plants that have been used for the snakebite include *Acalypha indica, Achyranthus aspera, Achyranthus superba, Capsicum annuum, Datura fastuosa, Strychnos colubrine, Rauwolfia serpentina, Hemidesmus indicus, Aritolochia radix, Mimosa pudica, Withania somnifera, Tamarandus Indica* and *Anacardium occidentale* (Chopra et al., 1958; Nadkarni, 1976; Sathyavathi et al., 1976; Gowda, 1997; Alam and Gomes, 1998; Mahanta and Mukherjee, 2001; Girish et al., 2004(a); Deepa and Gowda, 2002; Ushanandini et al., 2006, 2009). Many attempts are continuously being made in the direction of neutralization of venom toxicity using active principles isolated from various plants.

The current and most accepted therapy available until today for snakebite is the anti-venom therapy. This therapy drastically reduced the mortality rate caused by snake venom poisoning. Anti-sera against various kinds of snake venoms have been prepared and their effectiveness in treatment of snake venom poisoning has been widely accepted. Monospecific (prepared against single species of snake venom), bispecific (pre against two species of snake venoms) and polyspecific (prepared against mixture of selected species of snake venoms) anti-venoms are produced commercially by several laboratories all over the world (Theakston and Warrell, 1991). Although, the mortality due to snake venom poisoning is reduced markedly by the use of anti-venoms, there are several inherent drawbacks associated with it. Anti-venom therapy is less or offers no protection against morbidity caused by the snake venom poisoning and excess infusion of anti-venom increases the potential risk of serum sickness, which leads to arthritis, vasculitis and nephritis. The anti-venom prepared in India is mostly polyvalent using the venoms of

all the big four snakes. However, inter and intraspecific (Chippaux et al., 1991; Daltry et al., 1996; Sasa, 1999; Shashidhara murthy et al., 2002) venom variability offers serious threat for the efficacy of anti-venoms and the associated therapy.

Table. 1.1: Distribution of "Big Four" snakes in India

Common name **Scientific name**	**Family**	**Distribution**
Cobra *Naja naja*	Elapidae	Throughout India, sea level upto 4000 m (in the Himalayas)
Russell's viper *Daboia/Vipera russellii*	Viperidae	Hills and plains throughout India upto 3,000 m.
Saw-scaled viper *Echis carinatus*	Viperidae	Throughout India, sea level upto 2000 m.
Common krait *Bungarus caeruleus*	Elapidae	Throughout India, sea level upto 1700 m.

Table. 1.2: Plants active against venomous snakes (Soares et al., 2005 and references there in**).**

Plant family	**Plant species**	**Snake species**	**Antiophidian activity**	**Plant sources(s)**
Euphorbiaceae	*Acalypha indica*	*DR*	Lethality, hemorrhagic, necrotizing, cardiotoxic, neurotoxic	Leaves
Araceae	*Alocasia sp.*	*NN, NH, BM, Bat*	Hemorrhagic	Rhizomes
Apocynaceae	*Asclepias curassavica*	*Bas*	Hemorrhagic	Leaves
Asteraceae	*Baccharis sp.*	*Bal, BM, BJ, BN,*	PLA_2, hemorrhagic, proteolytic	Leaves
Bixaceae	*Bixa orellana*	*Bat, Bas, LM, Cdt, MM*	Lethality, hemolytic, edema, clotting and defibrinating	Leaves
Polygalaceae	*Bredemeyera floribunda*	*BJ*	Lethality	Roots
Fabaceae	*Brongniartia podalyrloides*	*Bat*	Lethality	Roots
Fabaceae	*Brownea rosademonte*	*Bat, Bas, LM, Cdt, MM*	Lethality, hemolytic, edema, clotting and defibrinating	Stembarks
Scrophulariaceae	*Buddleja nitida*	*Bas*	Hemorrhagic	Leaves
Fabaceae	*Caesalpinia bonduc*	*Bar*	Myonecrosis	Seeds and Leaves
Salicaceae	*Casearia marriquitensis*	*BN*	Coagulation	Leaves
Salicaceae	*Casearia sylvestris*	*Bas*	Hemorrhagic	Leaves

Meliaceae	*Cedrela tonduzii*	*Bas*	Hemorrhagic	Leaves, stems
Verbenaceae	*Citharexylum macrodenium*	*Bas*	Hemorrhagic	Leaves
Rutaceae	*Citrus limon*	*Bat, Bas, LM*	Lethality, edema, clotting, defibrinating	Ripe fruits
Asteraceae	*Clibadium Silvestre*	*Bat*	Hemorrhagic	Whole plant
Euphorbiaceae	*Croton draco*	*Bas*	Hemorrhagic,	stems
Zingiberaceae	*Curcuma sp.*	*Cdt, BJ, Bal, Nnd*	Lethality, hemorrhagic, proteolytic, neurotoxic, edema, necrosis	Roots, rhizomes
Ebenaceae	*Diospyros kaki*	*TF, LS*	Detoxification, lethality	Fruits, seeds
Araceae	*Dracontium croatii*	*Bat, Bas,*	lethality, edema, clotting, defibrinating	Rhizomes
Asteraceae	*Eclipta prostata*	*BJ, Cdt, Bja, Cvv, Acl, CR*	Lethality, myotoxicity, hemorrhagic	Aerial parts
Boraginaceae	*Ehretia buxifolia*	*EC*	Lethality	Roots
Euphorbiaceae	*Emblica officinalis*	*DR, NK*	Hemorrhagic, edema, lethality, coagulant	Roots
Apiaceae	*Eryngium creticum*	*CC*	Hemolytic	Leaves, roots
Moraceae	*Ficus nymphaeifolia*	*Bas, Bat*	Edema, hemorrhagic, defibrinating, coagulant	Leaves, branches and stems
Rubiaceae	*Gonzalagunia panamensis*	*Bas, Bat*	Hemolytic, edema, clotting, defibrinating	Leaves, stems
Combretaceae	*Guiera senegalensis*	*EC, NN*	Lethality	leaves

Fabaceae	*Harpalyce brasiliana*	*Bja*	Myotoxicity	Roots, compounds
Heliconiaceae	*Heliconia curtispatha*	*Bas, Bat*	Lethality, hemolytic, edema, clotting, defibrinating	Rhizomes
Apocynaceae	*Hemidesmus indicus*	*DR, NK, EC, OH*	Lethality, hemorrhagic, clotting, fibrinolytic, defibrinogenating	Roots
Asteraceae	*Lychnophora pinaster*	*Bal*	Edema, hemorrrhagic, necrosis	Leaves
Apocynaceae	*Mandevilla illustris*	*Cdt*	PLA_2, lethality	Roots, leaves
Lamiaceae	*Marsypianthes chamaedrys*	*Bj*	Fibrinoclotting	Whole plant
Fabaceae	*Mimosa pudica*	*NN, NK, DR EC*	Proteolytic, hyaluronidase, lethality, myotoxitcity	Roots
Cucurbitaceae	*Momordica charantia*	*Bat*	PLA_2, hemorrhagic	Whole plant
Moraceae	*Morus alba*	*BJ*	PLA_2	Stems and leaves
Fabaceae	*Mucuna pruriens*	*EC*	Lethality, coagulant, myotoxic, cytotoxic	Leaves and seeds
Musaceae	*Musa sp.*	*Bja, Cdt*	Myotoxic, PLA_2	Stems sap
Fabaceae	*Parkia biglobosa*	*Nni, EO*	Cytotoxicity, hemorrhagic, lethal	Stems bark
Araceae	*Penellia ternata*	*BJ*	Lethality	Rhizomes
Fabaceae	*Pentaclethra macroloba*	*Bat, Bmo, Bja, Bal, BJ, BN, BP, LM*	Lethality, hemorrhagic, nucleotidase, proteolytic	Barks

Lauraceae	*Persea americana*	*Bas*	Hemorrhagic	Seeds,
Phytolaccaceae	*Petiveria alliacea*	*Bas, Bat*	Hemorrhagic, PLA_2	Leaves, branches
Lauraceae	*Phoebe brenesii*	*Bas*	Hemorrhagic	Stems, compunds
Fabaceae	*Platymiscium pleiostachyum*	*Bas*	Hemorrhagic	Leaves
Polypodiaceae	*Pleopeltis percussa*	*Bas, Bat*	Lethality, hemolytic, clotting, defibrinating	Whole plant
Rubiaceae	*Schumanniophyton magnificum*	*NM, Nni, NK, EO, EC*	Myonecrotic, lethality, coagulant	Stem barks,
Cyperaceae	*Scleria pterota*	*Bja, Bmo, Bal, BN*	PLA_2, coagulant, hemorrhagic	Leaves
Asteraceae	*Solidago chilensis*	*Bal*	Edema, hemorrhagic, necrosis	Aerial parts
Verbenaceae	*Stachytarpheta jamaicensis*	*Bas*	Hemorrhagic	Leaves
Apocynaceae	*Strophanthus sp.*	*EC*	Blood clotting	Leaves
Loganiaceae	*Strychnos nux-vomica*	*DR, NK*	Lethality, hemorrhagic, defibrinogenating, PLA_2, cardiotoxicity, neurotoxicity	Seeds
Bignoniaceae	*Tabebuia sp.*	*Bas, Bat*	Hemorrhagic, lethality, edema, coagulant, hemolytic	Stems
Apocynaceae	*Tabernaemontana catharinensis*	*Cdt*	Lethality, myotoxic	Root barks
Lamiaceae	*Vitex negundo*	*DR, NK*	Lethality, hemorrhagic, coagulant, defribrinogenating,	Roots

			fibrinolytic, edema	
Solanaceae	*Withania somnifera*	*NN*	$PLA_{2,}$ myotoxicity, edema, cytotoxicity	Roots

Note: *CR = Calloselasma rhodostom, NK=Naja kaouthia, DR= Daboia/Vipera russellii, NN=Naja naja, BM=Bungarus multicinctus, Bat =Bothrops atrox, Bas= Bothrops asper, BJ=Bothrops jararaca, Bal=Bothrops alternatus, BM=Bothrops moojeni, Bja= Bothrops jararacussu, BN=Bothrops neuwiedi, LM= Lachesis muta, Cdt= Crotalus durissus terrificus, MM= Micrurus mipartitus, Bar=Bitis arietans, Nnd= Naja naja diamensis, TF= Trimeresurus flavoviridis, LS= Laticauda semifasciata, Cvv= Crotalus viridis viridis, Acl=Agkistrodon contortrix laticinctus, EC=Echis carinatus, EO=Echis oceliatus, CC= Cerastes cerastes, Nni= Naja nigricollis, OH=Ophiophgus Hannah, BP= Bothrops pirajai, NM=Naja melanoleuca.*

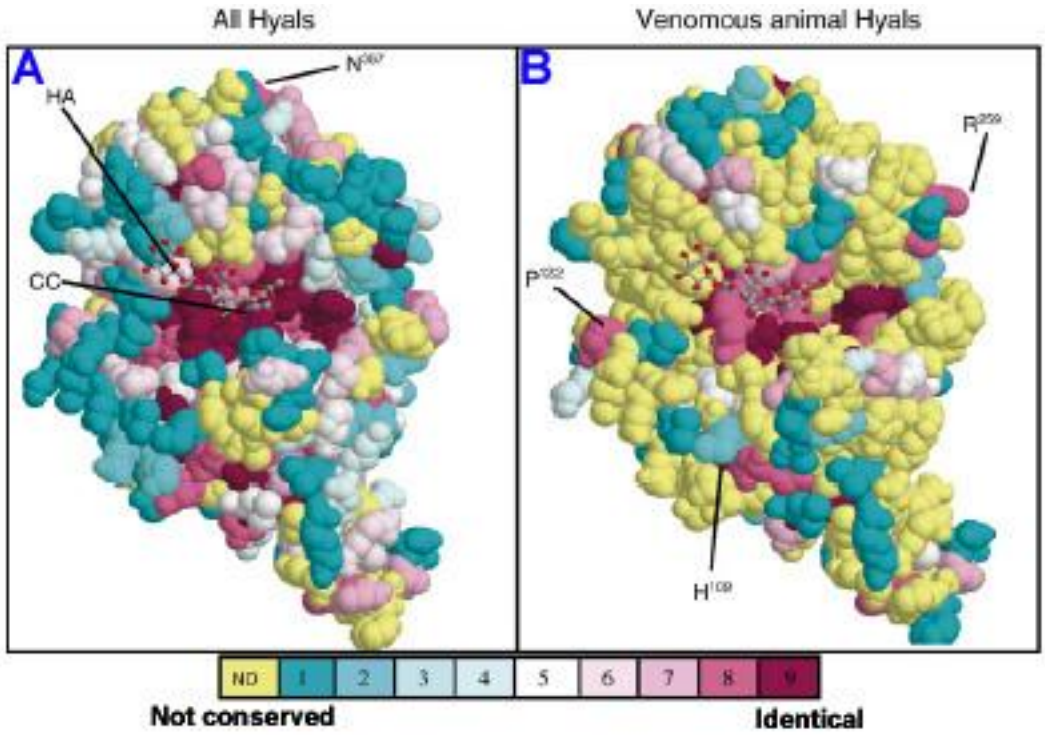

Fig. 1.1: Overlapping of simulated 3D structure of (A) mammalian hyaluronidase and (B) venom hyaluronidases over the crystal structure of *Apis mellifera* hyaluronidase. The images are oriented to show the catalytic cleft (CC) binding hyaluronan (HA). The key indicates colour coding of the extent of residue conservation (Harrison et al., 2007).

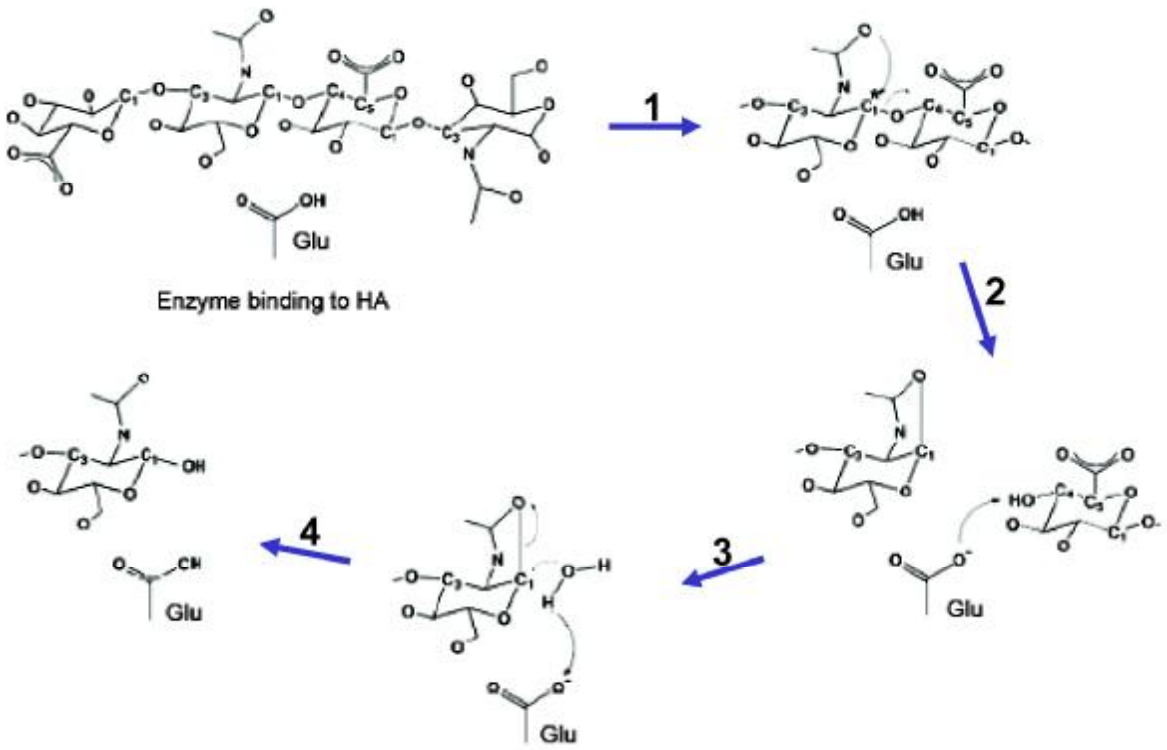

Fig. 1.2: Proposed catalytic mechanism of hyaluronan hydrolysis by snake venom hyaluronidase (Jedrzejas, 2000).

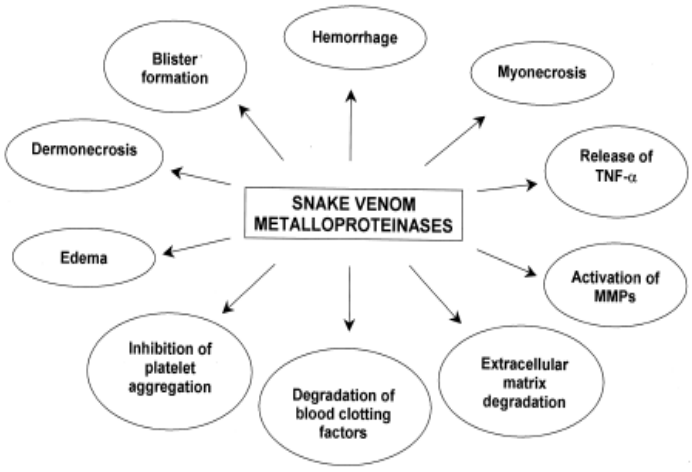

Fig. 1. 3: Biological activities of selected SVMPs (Gutierrez and Rucavado, 2000).

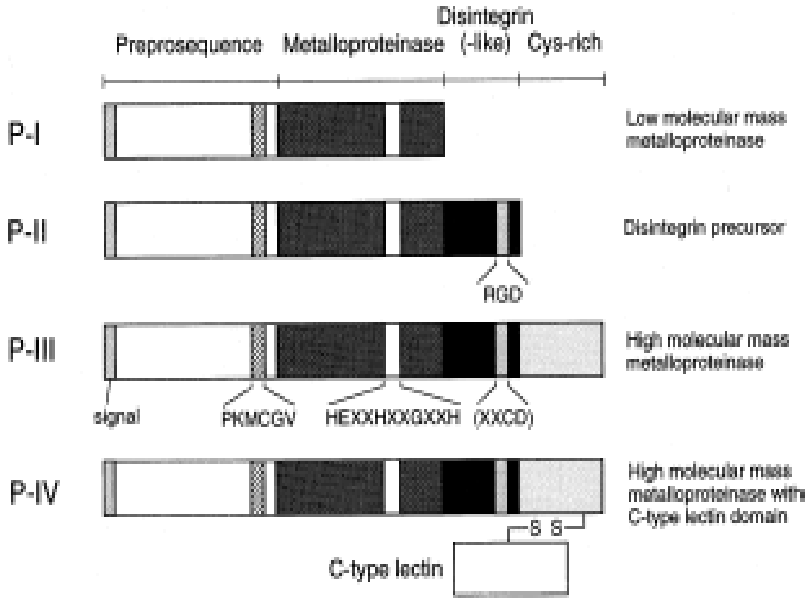

Fig. 1. 4: Schematic structures of snake venom metalloproteinases (Matsue et al., 2000).