Chapter 1: Introduction

Snakebites are an occupational and environmental disease. It is a common occurrence in tropical and subtropical countries (Warrell, 2010). It is common in agricultural countries affecting farmers and plantation workers leading to significant increase in mortality and morbidity. In 2009, World Health organization (WHO) has declared snakebites as a neglected tropical disease (Warrell, 2010; Gupta and Peshin, 2014). The precise number of deaths due to snakebite is not properly established and it continues to be a public health problem in most of the countries.

Joseph Fayrer quantified the snakebite deaths for the first time in 1869 for half of the British India (Including Pakistan, Bangladesh and Burma) and reported about 11,416 deaths. Many surveys have been carried out to assess global envenoming but estimate remains elusive. The analysis of snakebite mortality by Swaroop and Grab provided two interesting facts “primarily a considerable number of variations exist from one region to another and secondly the topographically similar regions were found to have higher rates of snakebite”. A total number of 30,000-40,000 deaths due to snakebite were witnessed worldwide excepting in China, USSR and Central European countries. The highest numbers were observed in Asia (25,000-30,000) followed by South America. North America, Europe and Oceania have reported low figures. In Africa, an exact number of estimates could not be provided, but the annual snakebite deaths were found to be 400-1000 (Swaroop and Grab, 1954).

Approximately, 12, 00, 000-55, 00, 000 snake bites occur globally with 2, 50, 000 envenomation and around 1, 25, 000 deaths annually (Chippaux, 1998). In 2008, Kasturiratne and colleagues reported 4, 21, 000-18, 41, 000 envenomings and 20, 000-94, 000 deaths. South and Southeast Asia, sub-Saharan Africa, Central and South America were found to have high number of incidences; India reported the highest number of bites (81,000) and deaths (11,000) for a single country (Kasturiratne et al., 2008).

**Figure 1.1**: Estimates of death due to snake bites worldwide
According to reports by Government of India, there were 61,507 cases in 2006 of which 1124 resulted in death. They also reported 76,948 bites and 1359 deaths in 2007, and 1364 deaths in 2008. It is believed that snakebite cases are underreported as many of them seek traditional treatment. 50,000 deaths in a year have also been reported (Warrell, 2010). In between 1974 and 78 an average of 1,224 deaths per year has been reported in the state of Maharashtra (2.43 deaths per 1,00,000 per year) and 16.4 deaths per 1,00,000 in West Bengal through some Random community based surveys (Hati et al., 1992). A survey in South 24 Parganas District of West Bengal has reported 4871 snake bites and 184 deaths in 2 years (Majumder et al., 2014). In Nepal 143 members were bitten by snake and half of them showed signs of probable envenoming (Sharma et al., 2004). Mohapatra et al., (2011) reported that a nationally representative mortality survey on snakebites in India (2001-2003) has highlighted 45,900 deaths annually, with the highest mortality rate in the state of Andhra Pradesh.

![Figure 1.2: Estimated deaths in India due to snake bite](image)

Snakebite influences their victims socially and economically. The envenomed person would need median dressing time of 10 days, is bed ridden for a minimum of four days and is physically unable to work for a minimum of seven days. The person has to personally spend USD 69 (Rs. 8479) for the treatment. This would decrease the income for the family if the victim works for daily wages (Sharma et al., 2004). A recent
survey conducted in some villages in Tamil Nadu reported that the transport and medical expenses would be around zero or little to 3, 50,000. The cost of the treatment will depend on the medical facilities availed. In order to pay the bills the victims’ family has to sell crops stored for the family or take loans or, in some situations discontinue their children from school. According to the Indian Labour Bureau “the average daily wage in India for agricultural occupations in 2007–2008 was Rs. 76 for a man and Rs. 54 for a woman”. This would limit them financially and affect the family livelihood (Vaiyapuri et al., 2013). In Nigeria the main source of revenue for most of the families is through agriculture as well. They are more prone to envenoming by snakes; the non-mechanised tilling of soil and farmers walking barefoot in the fields leave them exposed to snakes (Harrison et al., 2009). Hence snake bite is a serious problem associated with poverty in underdeveloped countries.

Mortality due to snake bite is due to shortage of anti venom supply/production, storage, cost, lack of education and poor treatment protocols. It also depends on the chance of a person being bitten by a snake which in turn depends on the prevalence of the poisonous snakes in those areas and also person coming in contact with them. For example person walking barefoot is known to have higher chance of being bitten when compared to a person with well protected limbs (Reid and Theakston, 1983). Also immediate medical help or first aid would lessen the burden of deaths. Hence knowledge based on the species and variations among snakes would definitely help in the treatment of snakebites.

1.1 Snakes

Snakes are carnivorous vertebrates distributed throughout the world. Of 3000 snake species known worldwide, 450 are potentially dangerous to humans. They belong to class Reptilia, Order Squamata and Suborder Serpentes (Warrell, 2010).

Venomous snakes are found in almost every country between latitudes 50°N and 50°S in the western hemisphere and 65°N (Scandinavia) and 50°S in the eastern hemisphere. Sea snakes are found in the Indian Ocean and Pacific Ocean between latitudes 30°N and 30°S, they are found more than 100 m depth in the oceans (Heatwole, 1999). On land, venomous snakes have been found from sea level up to altitudes higher than 4000 m in the Americas (Klauber, 1972) and Himalayas. The Integrated Taxonomic Information system has classified snakes into the infraorder Alethinophidia, superfamily Caenophidia, and further classified into four families: Viperidae, Atractaspidae, Elapidae, and Colubridae (Brutto, 2013).
1.1.1 Atractaspidae (mole vipers, burrowing vipers)

These are found mainly in Africa and the Middle East. This family has over 50 species, further classified into subfamilies. The species are recognized by their small size and by the varied characteristics of their fangs. Some species may be fangless or may have long fangs (Atractaspis aterrima). Most of the species of this family are harmless while the venom of Atractaspis engaddensis possesses potent lethal cardiotoxins called safarotoxins (Kochva et al., 1993; Kurnik et al., 1999).

Local effects include pain, swelling, blistering, necrosis, local numbness. The systemic effect is fever. In most of the cases the victims die within 45 min after bite due to vomiting, releasing excess saliva and lapsing into coma. The Atractaspis venom is highly toxic (Warrell et al., 1976) and the venom of A. engaddensis contain sarafotoxins that causes vasoconstriction and atrioventricular block. Their venom also possesses haemorrhagic and necrotic factors but no neurotoxins (Duncane, 2005).

1.1.2 Colubridae

These snakes are distributed all over the world except Antarctica and are mostly harmless to humans. They possess smaller size fangs that are positioned at the back of the mouth. (Warrell, 1993; Asmundsson et al., 2001). The boomslang (Dispholidus typhus), the vine snake (Thelothornis kirtlandii), and the Japanese garter snake (Rhabdophis tigrinus) (Kamiguti et al., 2000) are relatively dangerous species of the family.

The onset of envenoming is possible if colubrids engage their rear fangs for 15 s or more. The symptoms of bites include nausea, vomiting, abdominal pain and headache. Bleeding might occur from old or recent wounds and there is spontaneous gingival bleeding, epitaxis and haemorrhage. Most of the fatal cases succumb to renal failure that presents itself many days after the bite. Local effects are few but some show swelling. The clinical features include complement activation by alternative pathway, anaemia, thrombocytopenia (Nicoloson et al., 1974).

1.1.3 Elapidae

The snakes of this family are distributed over tropical and subtropical regions of the Americas, Africa, Asia, and Australia (Warrell, 1993; Enwere et al., 2000). These are also found in the Pacific and Indian Oceans known as sea snakes (formerly classified as a different family, Hydrophiidae). Some of the examples of Elapidae are mambas (Dendroaspis spp.), the kraits (Bungarus spp.), the cobras (Naja spp.), and the coral snakes (Micrurus spp. and Micruroides spp.). The size of snakes may vary from few centimetres to more than 5 meters in length. The size and shape of the head and fangs are also varied. As a defence mechanism, some snakes of this family have the ability to spit the venom from holes near the fangs. The coral snakes are found in the Southwestern USA and in the north of Mexico (Gold et al., 2002).
The colour of the coral snakes often resembles the non-venomous snakes Lampropeltis spp., while the distribution of the color bands on their bodies mark the difference.

The local effects after a bite are usually mild; however the African spitting cobras and Asian cobras commonly develop local swelling, which may be extensive and regional lymphadeopathy. Within 24 to 48 h, characteristic lesions can be observed with blistering around a demarcated black anaesthetic area of skin. Skip lesions, separated by normal skin may extend in the limbs. Severe envenoming by king cobra cause swelling of the whole limb but necrosis is absent (Warrell, 2009).

The first symptom of systemic envenoming is repeated vomiting, contraction of the frontalis, blurred vision, numbness around the mouth, loss of smell and taste, headache, vertigo, hypersalivation. Paralysis is observed as ptosis and ophthalmoplegia. These might appear as early as 15 min (cobras and mambas) or may delayed upto 10hrs after the bite (kraits). The facial muscles, palate, jaw, tongue, vocal cords, neck muscles may get paralysed. Respiratory blockage can occur due to the obstruction of upper airway by the paralysed mouth or due to inhaled vomitus (Warrell, 2009). Posterior reversible leukoencephalopathy can be an uncommon late neurological complication of common Indian krait bite (Kaushik et al., 2014).

1.1.4 Viperidae

These are distributed in Americas, Europe, Africa and Asia (Kerrigan, 1991; Enwere et al., 2000; Malina et al., 2008; Walter et al., 2009; Alirol et al., 2010). Some of the species are most dangerous to human beings namely Russell’s viper (Daboia russelli), the carpet viper (Echis carinatus), the puff adder (Bitis arietans), the rattlesnakes (Crotalus spp. and Sistrurus spp.), and the lance-head pit vipers (Bothrops spp. and Bothropoides spp.). The vipers are recognized by their long hollow fangs in front of their mouth, vertical pupils, and triangular-shaped head.

These produce more local effects than any other venom. Swelling appears within 15 minutes of the bite. It spreads immediately to the whole limb and trunk might swell (Reid and Theakston, 1983). Brusing is a common occurrence. Persistent bleeding from the fang marks might be seen. Necrotic skin, tissue and muscle develop in about 10% of the cases. The absence of the local swelling after several hours of the bite concludes that no venom has been injected (Warrell, 2009).

Hemostatic effects are characteristic of viperidae envenoming. Persistent bleeding from the bite site and old wounds would be the first sign of viperids bite. Blood pressure fall is a common occurrence as well. The pulse rate may be slow or irregular depending on whether it is affecting heart directly or reflexly. Vomiting, shock, sweating, bronchospasm, and oedema of face, lips, gums, and tongue may appear within
five min or later (Reid and Theakston, 1983; Warrell, 2009). Renal failure is the most frequent cause of death in case of Russell’s viper bite (Reid and Theakston, 1983; George et al., 1987). Neurotoxicity is due to the venom phospholipases. The features would be similar to that of elapid envenomning (Warrell, 2009).

1.2 Poisonous snakes in India

In India, 216 species are found out of which 63 are considered poisonous. The medically important “Big Four” poisonous snakes are distributed throughout the country and they are Indian cobra, common krait, Russell’s viper, Saw scaled viper (Whitaker and Whitaker, 2012). Recently, Ophiophagus hannah (King cobra), Hypnale hypnale (Hump nosed pit viper) and Macrovenipera lebatina (Levantine viper) (Fig1.3) are considered equally important with the increased number of bites by these species (Lekh et al., 2008).

**Figure 1.3**: Medically important snakes of India other than the “Big Four”

1.2.1 Bungarus caeruleus (Common Indian krait)

**Figure 1.4**: Bungarus caeruleus

It is about 1-4 feet long. It is ten times poisonous than cobra and most poisonous among elapids. It is a nocturnal terrestrial species and preys on rats and mice, commonly found near human dwellings. Their habitat is close to human habitation. The bites occur when the victim is sleeping on the floor, and he/she accidently crosses the snake during its hunt for prey. It accounts for about 30-35% fatal cases in the
subcontinent and most of the victims die due to lack of ventilator or improper management of the bite (Bawaskar and Bawaskar, 2015). An average yield of venom during bite is 20mg and the fatal dose to humans is about 6mg (Syed et al., 2008).

1.2.2 Naja naja (Indian Cobra)

It is distributed throughout India. The common cobra is a smooth scaled snake with black eyes, wide neck and head and medium body. Colouring varies from black or dark brown to yellowish white. The famous hood marking of the classic design shows a connected pair of rings (Whitaker, 2006). It is about 1m at birth and can grow maximum up to 2m. The common habitats are rice fields and some dry areas of country as well. They feed on insects, rats, frogs, lizards, toads. The bite generally occurs during day time and early darkness (Whitaker, 2006). Average yield of venom is about 60mg and fatality in humans occurs with a minimum of 12 mg (Syed et al., 2008).

1.2.3 Echis-carinatus (Saw scaled viper or carpet viper)

It is 1-3 feet in length. It is pale brown or tawny with dark brown in colour. It thrives in the hot and humid zones of subcontinent. It is viviparous producing 3 to 15 young at a time. Farmers, hunters, laborers
and people walking bare foot in rocky areas or often the victims of this species (Bawaskar and Bawaskar, 2015). Average yield of venom is 13-40 mg and it’s fatal for humans at 8 mg (Syed et al., 2008).

1.2.4 Vipera russelli (Russell’s viper)

This species of snake is responsible for most snakebite and envenoming in India, Sri Lanka, Burma and Pakistan. It is about 3-5 feet long. It survives on mice, rats, frogs, birds. They eat smaller snakes as well and are considered cannibalistic. Females give birth to 20-70 off springs around June or July (Bawaskar and Bawaskar, 2015). They are heavy, rough scaled and have a very bright pattern. The body colour is usually brown or yellowish (Whitaker, 2006). On average, this species delivers about 63 mg of venom and 15 mg of this venom in a bite can result in human death (Syed et al., 2008).

1.3 Clinical Features of Snake bite

1.3.1 Dry Bites

Some of the venomous snake does not introduce the venom into the system. Hence it is called dry bite (Warrell, 2009; Brutto, 2013). People (10-80%) bitten by venomous snakes with all the signs and marks would not result in envenomation. The amount of venom injected into the victim might not be controlled by the snake itself. It is a reflex reaction and a natural mechanistic phenomenon to capture its prey (Warrell, 2009).

1.3.2 Local effects

The presence of two puncture marks indicates bite by a poisonous snake. In case of a non–venomous bite small puncture wounds are in the form of an arc. Burning sensation and pain might develop immediately and shoot up in the bitten limb (Mehta and Sasidharan, 2002).

Local swelling: the increased vascular permeability and extravasation of plasma or blood might cause swelling and bruising. It might become apparent in 15 min and becomes massive in 2-3 days (Mehta and
Sasidharan, 2002). The venom endopeptidases, phospholipases, metalloproteinase, histamine serotonin cause this effect. Some species of Viperidae might cause pulmonary oedema, Facial oedema and haemoconcentrttration (Warrell, 2009).

**Local necrosis:** it occurs from the action of venom myotoxins and cytotoxins. This feature is apparent in viper bites. Cobra bites might cause local swelling and blistering. Krait bites does not produce any local features (Warrell, 2009). The microbial (Bacterial) flora present in the oral cavity of the snakes might cause secondary infection (Mehta and Sasidharan, 2002).

1.3.3 Systemic effects

Hypotension might occur with or without any features of anaphylaxis. This is probably caused by the release of autacoids. In viperids the oligopeptides inhibit angiotensin-converting enzymes (ACEs) and enhance the activity of bradykinin activating peptides. The viper bites also cause vasodilatation and a direct effect on myocardium resulting hypotension (Douglas, 1985).

Elapid and colubrid venoms activate blood complement system through alternative pathway, whereas the viperid venoms activate the classical pathway. This activation might affect the blood coagulation and humoral mediators (Hutton and Warrell, 1993; Vogt, 1990).

Anti- haemostasis is one of the features of envenoming by vipers, Australian elapids and colubids. These venoms cause incogulable blood; sometimes cause haemorrhage followed by snakebite. Often these are referred as haemotoxic or hemolytic. Most of the snake venoms are haemolytic in vitro, but it does not have any clinical significance. But envenoming by Daboia russelli, bothrops species some Australian elapids and colubrid members cause intravascular haemolysis resulting in renal failure (Hutton and Warrell, 1993).

Renal failure: It is a severe complication followed by even mild envenoming like in case of *Hypnale hypnale* (Sitprija and Chaiyabutr, 1999). It is a common occurrence in case of Russell’s viper bite resulting in death (Myint-Lwin *et al.*, 1985). Russell’s viper venom produces severe hypotension, nephrotoxicity and intravascular coagulation, intravascular haemolysis (Ratcliffe *et al.*, 1989). A number of histopathological changes have been described following a snake bite; distal tubular damage, proliferative glomerulonephritis causing nephrotoxicity and calcification (Sitprija and Chaiyabutr, 1999).

Neurotoxicity: the polypeptides and phospholipases block the transmission at neuromuscular junctions and cause paralysis. It is a common characteristic of envenomation by elapids, and cobras. The victims with paralysis of bulbar muscles might die of upper airway damage, but mostly they die of
respiratory paralysis after neurotoxic envenomination (Watt et al., 1986). Drowsiness might occur when elapids or viperids bite due to absence of respiratory or circulatory failure. This is due to release of endogenous opiates (Bevan and Hiestand, 1983).

Rhabdomyolysis: characterized by the release of myoglobin, uric acid, muscle enzymes into the bloodstream of victim by the action of phospholipase A\textsubscript{2}, presynaptic neurotoxins of most of the sea snakes (Warrell, 1994). The victims might die of bulbar or respiratory muscle weakness, acute hyperkalaemia or renal failure.

1.4 Snake venom

Snake venoms are one of the chemically complex venoms of all natural venoms. It is a cocktail of enzymes, non enzymatic proteins, carbohydrates, lipids, amines and other compounds (Kochva et al., 1993; Cintra-Francischinelli et al., 2010; Ohler et al., 2010; Vaiyapuri et al., 2010). The composition can vary depending on the geographical location, and within the population. It may also vary depending on the diet, age, season and environment (Gupta and Peshin, 2014). They typically possess 30 to over 100 protein toxins ranging from 6 to 100 kDa (Gold et al., 2002). The most common enzymes in snake venoms are phospholipase A\textsubscript{2}, acetylcholinesterase, hyaluronidase, nucleotidases, L- Amino acid oxidases, Serine proteases, metalloproteases (Kang et al., 2011).

The venom is produced and stored in venom apparatus, consisting of glands, compressor muscles, ducts and hollow fangs that are located behind and below the eyes of the snake. The venom injected to any human body varies and does not depend on the size of the snake or the fangs. Some of them do not introduce the venom into the system hence called dry bites (Brutto, 2013).

The snake venoms are mainly characterized as neurotoxic and hemotoxic. The neurotoxic venom disrupts neuromuscular junctions limiting muscle activity whereas the hemotoxic venom affects the circulatory system apart from body systems.

The venom components are mainly directed to immobilize and capture the prey except for enzymes like hyaluronidase which are involve in diffusion of other toxins. There are many other components of the venom which are responsible for clinical manifestations of envenoming (Doley and Kini, 2009).

1.4.1 5' - Nucleotidase

5'-nucleotidase (EC. 3.1.3.5) was originally discovered in the venom of snake and later on shown to be present in mammalian tissues and bull seminal plasma (Koshland and Springhorn, 1956). It is a
ubiquitous enzyme in snake venom having a major role in envenomation (Arid, 2002). It readily cleaves 5´ nucleotides and other phosphate esters slowly (Koshland and Springhorn, 1956). The primary function of this enzyme is liberation of adenosine and other purines and it also inhibits platelet aggregation (Arid, 2002).

1.4.2 Hyaluronidase

Hyaluronidase (E.C. 3.2.1.35), an endoglycosidase is an important component of the venom and often referred to as “Spreading factor”. It plays a key role in local envenomation. Hyaluronidase distorts the extracellular matrix of the local tissue by degrading the hyaluronan and evidently helps in spreading of other toxins. Otherwise it lacks toxicity (Girish et al., 2004b). It gains merit as it causes local tissue necrosis and results in permanent disfigurement or disability in victims. It helps in circulation of lethal toxins/venom components, and thus resulting in systemic effects. Oligosaccharides generated from the degradation of the hyaluronan may cause hemostatic disturbances (Kempraju and Girish, 2006). It has been purified from various diverse sources such as stone fish, bee, wasp, freshwater, spider, scorpions, and from snakes (Girish and Kempraju, 2007).

1.4.3 Acetylcholinesterase

Acetylcholinesterase (AChE, EC.3.1.1.7) plays an important role by inactivating acetylcholine neurotransmitter. It belongs to cholinesterase family and hydrolyzes choline esters. Snake venoms AChE was first discovered in 1938. They are found in Elapidae but not in Viperidae and Crotalidae Venoms. The studies of snake species has found that the AChE in Bungarus species have high activity (> 60,000 Ellman units/mg). Recent literature has shown that it is present in some non venomous snake as well (Mackessy et al., 2006).

1.4.4 Phosphodiesterase

Phosphodiesterase (EC 3.1.15.1) is mainly present in viperids with varying levels of activity. The Elapids have lower levels of this enzyme and in some as *Bungarus* there is negative activity (Aird, 2002). It liberates 5´- mononucleotides from the 3´ end of polynucleotides that ensures enough substrate supply for venom 5´- nucleotidase (Iwanga and Suzuki, 1979). It hydrolyzes both DNA and RNA, native DNA is a better substrate than the denatured ones. It also hydrolyzes ATP, ADP, FAD and other nucleic acid derivatives (Aird, 2002). Russell et al., (1963) reported that this enzyme causes profound hypotension in the victim.

1.4.5 Phospholipase A₂ (PLA₂)

These enzymes catalyze hydrolysis of sn-2 ester bond in glycerophospholipids to give lysophospholipids and fatty acids. These are 14-18 kDa proteins with 5-8 disulphide bonds. This family of
enzymes are grouped into 16 groups and several subgroups (Schaloske and Dennis, 2006; Duncan et al., 2008). The elapids and viperids possess GI and GII secretory PLA$_2$ respectively. They share 40-99% amino acid sequence similarity but display different pharmacological effects that include neurotoxic, cardiotoxic, myotoxic, tissue damaging activities and many others. Because of the wide spectrum of activity it targets specific tissues or organs by binding to the glycol receptors on the cells (Kini, 2003).

1.4.6 Phosphomonoesterases

Alkaline and acidic phosphomonoesterases are observed in the snake venom. The alkaline phosphomonoesterase is widely distributed. It is involved in dephosphorylating 5’ AMP, 5’ dAMP, ATP, FMN, NMN and other nucleotides (Aird, 2002). It is been found that the Elapid venoms contain more alkaline form than the viperid forms. The action of these enzymes results in the liberation of purines and pyrimidine nucleotides. The generation of adenosine is pharmacologically important as it causes snake envenomation symptoms (Dhananjaya and D’souza, 2010).

1.4.7 Proteases

There are more than 150 proteases so far structurally defined from the inventory of snake venom proteases. These proteases are found in most of the species except for hydrophidae. These are invariably classified into either serine proteases or metalloproteases. However relatively little evidence is available about thiol and aspartic proteases (Matsui et al., 2000). The proteases act on the specific target tissues or molecules to provide specific potent effects. It activates or inhibits hemostasis or thrombosis like blood coagulation, fribinolysis or platelet aggregation (Markland Jr., 1991; Markland Jr, 1998; Pirkle and Theodor, 1990; Pirkle, 1998; Pirkle and Stocker, 1991).

1.4.7.1 Serine proteases

These are present in venoms of Viperidae, Elapidae, and Cloubridae. Some of serine proteases have both fibrinogenolytic and fibrinolytic activities. Most of them possess fibrinogenolytic activity hence called thrombin like proteases (Matsui et al., 2000). Other proteases act like mammalian kallikrien releasing bradykinin from kiniongen hence called kallikrien like proteases (Bjarnason et al., 1983).

Thrombin like serine proteases release either fibrinopeptide A or B to form abnormal fibrin clots formed by short polymers and not cross linked by the activated factor XIII. This disrupts the blood coagulation system of the victim. This enzyme does not possess any other activating characteristics like that of mammalian thrombin (Matsui et al., 2000).
Kallikrien like serine proteases release bradykinin which causes hypotension into the mammalian plasma. An enzyme called hyalatase has been recently isolated from the venom of *A. halys blomhoffi*. It cleaves the fibrinogen at Bβ chain and then slowly degrades the Aα chain resulting in abnormal clots which no longer be converted into normal fibrin clots by thrombin. This group of enzymes causes reduction in blood pressure and also inhibits the blood coagulation (Matsui *et al.*, 1998).

**1.4.7.2 Metalloproteinases**

These are abundantly present in viperid and smaller quantities in elapid and colubrid venoms (Moura-da-Silva *et al.*, 2007). They are highly toxic interfering in the blood coagulation or the hemostasis of the system. They are all Zn$^{++}$ dependent metalloproteinases (Matsui *et al.*, 2000). This group of enzymes have hemorrhagic, edema, hypotensive, necrotic activities (Gutiérrez and Rucavado, 2000).

**1.4.8 L-Aminoacid oxidase**

L-Amino acid oxidase (E.C. 1.4.3.2.) is a flavoenzyme that catalyzes the oxidative deamination of an L-amino acid to form the corresponding α-ketoacid and ammonia (Tan and Fung, 2008). They are the major components in venom forming approximately 30% of the snake venom of some species. The venom is yellow in colour and postulated to be toxins. The enzymes have molecular mass of 110-115 kDa and have affinity for the hydrophobic amino acids like Leucine (Zuliani *et al.*, 2009).

**1.4.9 Neurotoxins**

The snake venom possesses certain proteins that affect prey’s nerve function, by causing convulsions or paralysis. They are subdivided into two group’s firstly non enzymatic postsynaptic neurotoxins or α-neurotoxins which block neurotransmitter receptors, and secondly presynaptic neurotoxins that inhibit or cause release of neurotransmitter. The post synaptic toxins are of two types short and long chain consisting 60-62 amino acids with 4 disulphide bonds and 66-74 amino acids with 4-5 disulphide bonds respectively. More than 100 post synaptic neurotoxins have been isolated having low molecular weight (6-8 kDa). The presyanptic toxins are mostly phospholipases A$_2$ and they exert the same effect as that of the enzyme (Stocker, 1990).

**1.4.10 Cytotoxins**

These are polypeptides having 60-62 amino acid residues with four intramolecular disulphide bonds. The cytotoxins exhibit toxicity and some of the actions include hemolysis, cytolysis, depolarization of muscle membrane and cardiotoxicity (Stocker, 1990). Approximately 59 toxins have been isolated from Elapidae venoms (Mebs, 1985).
1.4.11 Myotoxins

Myotoxins are categorized into two groups. First group of myotoxins are polypeptides having 42-50 amino acid residues with 3 intramolecular disulphide bridges (Mebs, 1985) and possess approximately 25% lysine residues. They induce skeletal muscle degeneration resulting in prey digestion (Stocker, 1990) but the primary biological activity of them is paralytic. The second group of myotoxins are basic phospholipases lacking the catalytic activity due to the presence of Lys residue instead of Asp residue at position 49 of the calcium binding loop (Aird, 2002).

1.4.12 Cardiotoxins

These are membrane toxins causing contraction and depolarization of skeletal and cardiac muscle (Harvey et al., 1982; Fletcher et al., 1991). Cardiotoxins along with phospholipases A2 causes hemolysis resulting in K+ liberation causing cardiac arrest (Karlsson, 1979). These are widely distributed in elapids. They form the major constituents of the cobra venom almost 50% of the total venom protein (Aird, 2002). Cardiotoxins, sarafotoxins have been isolated from the venom of Atractaspis engaddensis. Sarafotoxins S6 is one of the Cysteine rich, acidic, 21-residue polypeptides (Stock, 2000).

1.5 Antivenom

The most effective antagonist of snake venom is the anti snake venom (ASV). It is the F(ab) fragments of IgG purified from the serum or plasma of a horse, donkey or sheep that has been immunized with the venom of one or more species of snakes (Whitaker and Whitaker, 2012; Gupta and Peshin, 2014). Albert Calmette’s use of serum antivenom in 1895 was put to practice for treating envenomations with proper clinical trials (Bon, 1996). It neutralizes the toxicity of a particular species (monovalent/monospecific) or various different species (polyvalent/polyspecific). The antibodies against a particular species may also neutralize the venom of a closely related species (paraspecific activity) (Gupta and Peshin, 2014).

In India, the polyvalent anti snake venom was produced by hyper immunizing horses against the venom of four common poisonous snakes the “Big Four” (Cobra, Krait, Russell's viper and Saw-scaled viper), to produce. The venom is mostly procured from Chennai in South India (Whitaker and Whitaker, 2012). Some of the pharmaceutical laboratories in India that produce ASV against four medically important Indian snake species are Central research institute, Serum Institute of India Limited, Haffkine Bioharmaceutical Company Limited, VINS bioproducts Limited, King’s Institute of Preventive Medicine, Biological E. Limited, Bengal Chemicals and Pharmaceuticals Limited and Bharat Serum and Vaccine Limited (Syed et al., 2008).
WHO recommends antivenom to be produced in liquid form if the cold chain is properly maintained, as it reduces costs and decreases physiochemical changes brought by lyophilization. The development is a costly and time consuming process (Syed et al., 2008). The liquid form of ASV has a half life of 2 years. Storage at 0-4°C is necessary, otherwise leads to deterioration and becomes unfit for use. The lyophilized form has a shelf life of 5 years if stored in ideal conditions (Gupta and Peshin, 2012). Mostly it is supplied as dry powder to be reconstituted in 10 mL of normal saline by mixing.

During envenomation, antivenom should be administered within 4 h of bite; however it is effective within 24 h. It is administered by slow intravenous injection (2 mL per minute) or i.v. infusion in isotonic solution over a period of 30-60 min. The initial dose depends on the type of antivenom, species of snake and clinical effects of the bite. The doses are repeated during non reduction of life threatening bleeding, paralysis, shock or if the blood remains incoagulable when tested after six hours (Warrell, 2007). The dosage of antivenom has not been standardized through any clinical trials, World Health Organization recommends the dose of antivenom as the amount required to neutralize the average venom yield when captive snakes are milked for their venom (WHO, 2005). Research has indicated that on an average Russell’s viper inject 63 mg per bite hence 8-10 vials are required for initial neutralization (Syed et al., 2008).

The antivenom needs to be administered only when there is following indications (Warrell, 2007)

1. Spontaneous systemic bleeding.
2. The patient’s blood becomes incoagulable to clot when placed in a new, clean, dry, glass tube and left undisturbed for 20 minutes.
3. Raise or fall of blood pressure or cardiac arrhythmia
4. Paralysis
5. Blackness of urine due to the presence of blood/haemoglobin/myoglobin (indicating rhabdomyolysis or haemolysis)
6. The swelling of fingers and toes or the bitten limb or the local swelling after bite that might cause necrosis

1.5.1 Issues

The production of ASV started 100 years ago in India. The important issues with ASV in practice are species specificity, non availability, ideal storage conditions and affordability. The principal drawback of the serum therapy is the specificity. There is huge species variation as there are one Russell’s viper, four cobras and eight species of kraits; two subspecies of saw scaled vipers are identified. Moreover Russell viper also exhibits regional variation. Hence the composition variability and antigenic reactivity of the venom restricts the use of ASV. The production of ASV for all these species is not possible as the information regarding their snake venom is scarce (Whitaker and Whitaker, 2012; Kumar and Sabitha, 2011). The Hump nose pit
viper bite caused a death and two late recoveries indicating the inadequacy of antivenom in treating the victim (Kumar and Sabitha, 2011).

The Wildlife Protection Act has indicated that snakes cannot be collected or venom cannot be extracted without the permission from wildlife authorities. Hence it has resulted in conservative stance from the authorities to permit the capture of snakes in large numbers which has led to the decrease in antivenom production (Whitaker and Whitaker, 2012). The people for ethical treatment of animals (PETA) have raised their concern for using animals for the production of antivenom. The ideal storage conditions are not available in the rural areas hence the antivenom might not reach the victims in need.

The producers of antivenom have raised concern over the higher venom prices. The cost of krait is USD 888, saw scaled viper is USD 1000, Russell’s viper is USD 666 and cobra is USD 511 per gram in 2010 by a report released by Irula snake catcher’s Industrial cooperative society (ISCICS, 2010). The other issues include venom sourcing from unlicensed producers, non compliance of WHO guidelines for the production of venom and antivenom. The export of antivenom from India to countries like Africa and Papa New guinea has raised serious issues on production of antivenom (Warrell, 2008).

The scarcity of antivenom is another major concern, in Africa the French drug firm Sanofi Pasteur has ceased the production of antivenom and the stock will run out by the end of 2016. In India major cities like Bangalore, Kanpur and Kerala are not having adequate supply of antivenom to treat the victims.

India produces the cheapest antivenom USD 10 per vial. The range of venom yields for cobras reported in one study was 58–742 mg, which translates to a need for 13–165 vials (a treatment cost of USD 130–1,650 or Rs. 6,500–82,500). Another study in northern India reported the average number of vials used for bites by elapid snakes (kraits and cobras) as 90, for a treatment cost of USD 900 or Rs. 45,000. This can be quite expensive for an average wage worker.

1.5.2 Side effects of antivenom

Antivenom treatment is associated with early and late reactions

Early reactions: the complement system is activated by aggregates if IgG or its fragments. Reactions usually develop 10 to 18 h after the introduction of antivenom. Urticaria, itching, fever, tachycardia, palpitations, cough, nausea and vomiting are the common features. The occurrence of symptoms might vary from 3% to 34% and might increase with the dose of antivenom. The victim might die if the symptoms are not observed properly for reactions. Many of the patients show system anaphylaxis features like bronchospasm, hypotension (Mathivani et al., 2013; Warrell, 2012).
Pyrogenic reactions occur when the antivenom is contaminated with other compounds. Fever occurs after 1-2 h of administration of the antivenom; rigors develop followed by vasodilatation and fall in blood pressure (Mathivani et al., 2013).

Late or serum sickness reactions: they are observed after 5-24 days after treatment. The symptoms include fever, urticaria, itching, lymphadenopathy, albuminuria and rarely encephalopathy. Most of the late reactions are under reported as patients would have left the hospital (Mathivani et al., 2013; Warrell, 2012).

1.6 Herbal antidotes

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 (Alam, 2014).

Medicinal plants form local heritage with global use. The medicinal plants were used throughout human history, and the knowledge has been passed onto many generations which represents millenia of popular wisdom. In this regard, plants have contributed for the development of many drugs which are still in use, such as morphine, the main anaesthetic alkaloid in opium; vincristine, a antitumor compound, or rutin, a potent vasodilator apart from them using as folk medicinal plants (Soares, 2005).

A large number of plants were claimed to neutralize the action of snake venom. In 2005, Soares reported that 850 species of higher plants from 138 families were found in literatures that were used against snakebite. Due to the non availibitlity and side effects of antivenom, the alternative treatment using plants has caught attention of the scientific community in the recent 20 years. Many plants are been used by traditional healers and the tribal groups to treat snake bites. Ayurvedic medicine also mentions about the use of plants in treating snake bites.

Generally an aqueous/ methanol/ethanol extract were prepared out of plant or plant material. It is applied directly on the bitten area or made to chew leaves/ barks or made to drink extracts or decoctions of the extracts to antagonize the activity of the venom. *Abrus precatorius* root extract was used against Krait bite, *Casearia sylvestris* leaves and bark were used as a standard medicine to treat snakebite in Colombia and Asia. The juice of *Origanum dictamnus* was consumed in wine to treat snake bites (Gomes et al., 2010).
Many such plants have also been mentioned in Ayurvedic system for treating snake bites. Recently, the Pinak medicine produced by Shree Bharadi Ayurvedic pharmaceuticals was tested for its efficacy on the snakebite victims of Narodi village, Maharashtra. The drug is a unique combination prepared from four medicinal plants *Erythrina indica, Mangifera indica, Eugenia jambolana* and *Jusminum sambac*. The hospital records prove that the 22 victims treated with Pinak before taking to the hospital survived from envenomation (Raut et al., 2013).

In the present study the *Azima tetracantha* Lam and *Carissa spinarum* Linn extract/s were evaluated for their inhibitory effect on the *Vipera russelli* and *Bungarus caeruleus* venom through *in vitro* and *in vivo* methods.

### 1.6.1 *Azima tetracantha* Lam

![Azima tetracantha Lam](image)

**Figure 1.8:** *Azima tetracantha* Lam

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Tracheophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Capparales</td>
</tr>
<tr>
<td>Family</td>
<td>Salvadoraceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Azima</td>
</tr>
<tr>
<td>Species</td>
<td><em>Azima tetracantha</em> Lam</td>
</tr>
</tbody>
</table>

The spinous shrub flowering throughout the year was known as Kundali in Ayurvedic medicine, “Mullusangu” locally and uppimullu in Kannada. It has been enlisted as medicinal plant of value in ENVIS centre on medicinal plants. It was distributed in Peninsular India, West Bengal, Orissa, African countries which extends through Arabia to tropical Asia. The root bark was used in treating rheumatism and the juice of leaves was used to treat tooth and ear aches.
In East Africa the pounded roots of *Azima tetracantha* are applied directly to snakebites and an infusion was taken orally as a treatment for them, while in Zimbabwe a mixture of roots and leaves was used similarly (Hema et al., 2012; Hiremath and Tarranath, 2010). The Bajun people of the Kenyan coast use a root decoction to treat stomach disorders. In Madagascar an infusion of the leaves is used to treat venereal diseases (Hema et al., 2012). Antimicrobial, antioxidant and free radical scavenging, anti inflammatory, analgesic, antipyretic, hepatoprotective and antiulcer activities by the plant and their parts have been reported (Sundaresan and Ramalingam, 2015). It is also used for treating stomach disorders, venereal diseases, ear pain, tooth ache and rheumatism (Gayathri et al., 2012).

The aqueous leaf extract of *Azima tetracantha* was found to reduce the kidney damage induced by ferrous sulphate in rats (Manikandaselvi et al., 2012). The leaf extracts were found to possess free radical scavenging properties (Hepshibha et al., 2010). The hexane extract of the plant was found to possess antifungal activity (Duraipandiyan and Ignacimuthu, 2011). The antidiarrheal activity has been reported in this plant, antimicrobial activity in fruits. The presence of dimeric piperdine alkaloids azimine, azacarpaine, carpaine, triterpenoids, isorhamnetin 3-rutinoside, neoascorbinogen and glucosinolates and novel fatty acids have been reported as some of the phytochemicals present in the plant (Ekbote et al., 2012).

### 1.6.2 Carissa spinarum Linn

![Carissa spinarum linn](image)

*Figure 1.9: Carissa spinarum Linn*

**Kingdom** : *Plantae*
**Phylum** : *Tracheophyta*
**Class** : *Magnoliopsida*
**Order** : *Gentianales*
**Family** : *Apocynaceae*
**Genus** : *Carissa*
**Species** : *spinarum*
It is known as Karekayi/Karanda in Kannada. It has been enlisted as medicinal plant of value in ENVIS centre on medicinal plants. The plant has been widely distributed throughout the dry, sandy and rocky soils of India, Ceylon, Myanmar and Thailand. In traditional system of medicine the plant is used as purgative, for the treatment of rheumatism, cleaning worm infested wounds of animals and in snake bite. The roots act as a repellent in the case of snakes, when the roughly ground powder of the roots mixed with water is poured into the holes of snakes (Parmar and Kushal, 1982). Plant and their parts were used to treat wounds in cattle, rheumatism and pain (Hegde and Joshi, 2010).

The ethanolic extract of *C. spinarum* roots reduced the arthritic edema in the adjuvant induced arthritis in rats (Hedge *et al.*, 2010). The pyrexia induced by Brewer’s yeast in Albino Wistar rats was reduced dose dependently by the ethanolic extract of the root (Hegde and Joshi, 2010). The methanolic and chloroform extract showed antihelmintic activity on *Pheretima posthuma* (Harwansh *et al.*, 2010). The methanolic extract of the root has showed significant wound healing capacity evident by the rate of wound contraction and epithelisation (Sanwal and Chaudhary, 2011). The methanolic extract of leaves has shown hepatoprotective effect against the CCl₄ induced hepatotoxicity in rats (Khan *et al.*, 2011). The fruit, root and stem extracts have shown to possess antioxidant activities (Fatima *et al.*, 2013).

Previous studies on the roots of the *C. spinarum* has revealed the presence of several cardiac glycosides, caffeic acid, ursolic acid, naringin germacrane sesquiterpene and lignans were reported from this plan (Hegde aand Joshi 2010). The leaves were found to possess triterpenes, alcohol and urosolic acid (Fatima *et al.*, 2013).