Chapter 2

REVIEW OF LITERATURE
Section 1

THE SNAKES
Snakes are one of the most worshipped, adored and feared animals. Snakes have appeared throughout legend and history in one way or another. The snake is an especially recurrent theme in religious beliefs, legends, and ceremonies. It is a symbol of mysticism and power. It finds its presence in the Holy Bible, where it is mentioned that “be wise as serpents and innocent as doves”. It was the serpent who presented the temptation of the “sin” in the Garden of Eden. There are cobra cults in India, and in ancient Mexico Quetzalcoatl was the feathered serpent of the Aztecs. Snakes were kept in the temple of “Aesculapias” the Greek God of Healing. According to Egyptian belief, the Greek god of medicine, Aesculapias (Asclepius in Latin), appears in art holding a staff with a serpent coiled around it. The serpent, which was sacred to him, symbolized renewal of youth because it sheds its skin. Cleopatra, a queen of Egypt in the first century B.C., was famous for her beauty, charm, and luxurious living. She lived for some time in Rome with Julius Caesar. For several years after Caesar was assassinated, she lived in Egypt with the Roman politician Mark Antony. Antony killed himself on hearing a false report that she was dead. After Antony’s death, Cleopatra committed suicide by allowing an asp, a poisonous snake, to bite her. In Sri Lanka, Buddha is protected by the shadow of Cobra with seven heads. The cobra is widely worshipped by the local people. In Buddhist literature, Sanghamitra, the daughter of Emperor Ashoka, is shown chasing away the magic power of the nagas (cobras). The Koreans consider the serpent as the guardian of genii of their household. The sun and the serpent (Dragon or naga) are worshipped all over China and its neighbouring countries. In Greek mythology, Apollo killed several snakes. He was the god of light, youth, beauty, and prophecy. One of the earliest deeds of the young Apollo was the slaying of the deadly serpent Python. No other man dared to approach the beast. When the people asked Apollo to save them, he came down from
Mount Olympus with his silver bow and a quiver of golden arrows and killed the serpent. In Italian folklore, Bensurdatu slayed two giants and a serpent in “The Story of Bensurdatu.” The goddess Hera, who hated the infant Hercules, sent two serpents to destroy him in his cradle. However, Hercules strangled them. Later he slew the Hydra, a terrible serpent with nine heads. The basilisk, or cockatrice, was a serpent so horrible that it killed with a glance. Pliny the Elder described it simply as a snake with a small golden crown. By the Middle Ages it had become a snake with the head of a cock or sometimes a human head. It was born of a spherical egg, laid during the days of Sirius, the Dog Star, by a 7-year-old cock. The egg was then hatched by a toad. The sight of a basilisk was so dreadful that if the creature saw its own reflection in a mirror it would supposedly die of fright. The only way then to kill it was to hold a mirror before it and avoid looking at it directly. The origin of the basilisk could have been a horned adder or the hooded cobra of India.

In India, snake is an important symbol of religion, folklore and culture. The cobra is widely worshipped on Nag Panchami and Anant Chaturdashi. The serpent symbolizes power, aggressive forces of the gods of darkness especially in the tantras. In the Hindu religion, the Upanishads record the story of Kalianag, the king of serpents. Lord Krishna, as a child was saved by the serpent, who later demanded that he should worship the serpents. This angered Lord Krishna and he violently cut off the snakes head. Another legend has it that the coiled serpent ‘shesh nag’ was used as the churning rope by the Devas (God) and Ashurmas (Demon) to churn the ocean, the legend popular as ‘Samudra Manthan’. A number of temples have been dedicated to cobra, Sheshnag, Basaknag and Takthanag, in the Kangra Valley in North India. Lord Shiva is depicted as wearing a cobra round his neck. In Mahabharata, Arjuna was to marry the sister of Naga king Vasuki. In the Vedas, particularly the Rigveda, the king of Gods-Indra is believed to have killed Ahi-Vritrasura, who is said to be a snake demon.

Many beliefs are there in different religions which are not true. For example, it is believed that some snakes (Red Sand Boa) have a head at each end and they travel in both the direction; snakes possess a priceless jewel on their head; snakes, particularly the Cobra are fond of consuming milk, specially...
on Friday. In North West India, Kraits take away the breath of a sleeping person. This is basically due to the respiratory paralysis caused by a Krait bite (Whitakar, 1978). A small snake of Kashmir is so deadly that it melts the snow it passes.

Many diseases in India are believed to be contracted by the breath of the snake. In part, this could be derived from the venomous bite of the cobras and vipers. When first bitten by a venomous snake such as a common Krait (*Bungarus caeruleus*), the Slender Coral snake (*Callophis melanurus*), Indian Spectacled Cobra (*Naja naja naja*), Indian Monocled Cobra (*Naja kaouthia*), King Cobra (*Ophiophagus hannah*), Russell’s Viper (*Vipera russellii*) and the Saw-Scaled Viper (*Echis carinatus*) (Vogel, 1926) bite victims report an intense burning sensation before the onset of numbing and swelling (Whitaker, 1978). This phenomenon of intense burning due to venom, an occurrence which is very real in both ancient and modern India, is rationalized as a magical affliction.

Large amounts of folklore and superstition revolve around the venom of the snakes, as it is a constant threat for the residents of India. Of course, both the ancient and modern scientific community is also concerned with snake venom. The knowledge of snake venom and its antidotes is one of the eight primary topics of study in the Vedic medical texts (Vogel, 1926). Like many cultures believe that the cure is also the cause. An example of this is a tale from the Panchatantra, which describes a blind man recovering his sight from drinking the venom of a snake boiled in milk. Milk has strong representations of purity in the culture which accounts for the healing power of this story. Moreover, most snake venom causes blindness; either temporary or permanent. As we know now, this cure is not completely rooted in fantasy. Injecting a variety of venoms into a horse and extracting the antibodies from the horse’s blood creates commonly used anti-venom which is found in hospitals. Therefore using venom to cure venom is real. Another common ‘cure’ for snakebites in India is the ‘snake stone’. It is usually a bone soaked in blood and baked many times, it is believed to draw out the poison. It is not uncommon to find people still carrying these snakestones with them, especially in Thailand.

It is well known that families in India will welcome snakes into their household and consider it a sign of luck and prosperity. The family will give
the snake offerings of milk, bananas and other food items believed to be loved by the snakes. The truth is that all snakes that reside in India are only carnivorous and would not even eat rodents that were pre-killed. The family will often treat the snake as a guest, indicating great respect and awe for the snake. The snakes are useful when transported to the plantation fields because they eat the rodents that destroy the crops.

The Buddhist legend says that the pattern on the skin was given to the Naga King Muchilinda who sheltered Buddha for seven days by spreading his hood over the master’s head. Another belief is that the pattern is the footprint of Krishna on the head of Naga Kaliya (Snellgrove, 1987). Unfortunately, it’s the pattern on the hood of the Cobra that makes its skin so desirable to consumers both abroad and domestically.

Although the King Cobra is not as common in India as many other venomous snakes, they are one of the more unique. Knowledge and rumours about the King Cobra spread vastly even in ancient times. They are the only known snake in the world to build a nest and ‘behave with an intelligence and awareness unusual in snakes’ (Whitaker, 1978). Romulus Whitaker describes the King Cobra as a snake that would choose a quick flight over aggression any day, which is extremely contradictory to its reputation. King Cobras are also the worlds largest known venomous snakes reaching a common length of 3 meters, with the largest recorded was 6 meters in Thailand (Whitaker; 1978). Knowing these facts, it is easy to understand how snake’s powers became distorted and inflated in mythology.

Sesha is another Cobra (Naga) that plays a fundamental role in Hindu mythology. In the Harivamsa, Brahma appoints three Naga kings; Vasuki is appointed king of the Nagas, Takshaka king of the snakes and Sesha kings of all fanged beings. Sesha is also the well known Naga that Vishnu reclines on as seen in a wide variety of modern Indian plastic art. Sesha plays a fundamental role in the creation story; when Vishnu falls asleep and Brahma is born from the lotus flower rising from Vishnu’s naval the demons, Madhu and Kaitabha threaten the newborn Brahma. It is Sesha that defends Brahma each time so that he can create the universe that we know. Sesha also goes by the name

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Ananta ‘the Endless One’. In the Mahabharata there is a story where the devas have decided to churn the oceans to ‘drink of immortality’, they mix everything except Mount Mandara and out of frustration they turn to Vishnu and Brahma for help. Vishnu and Brahma command King Ananta to lift the mountain ‘together with the forests and the beasts’ for the devas.

The presence of the Nagas in Buddhist lore is abundant. In the Lalitavistara, Queen Maya stopped in the Lumbini Gardens to give birth. There, two Nagarajas appeared in the form of Cobras and produced two streams, one cold and warm, to bathe the newly born future Buddha. In the prophecy of the decline of Buddhist faith, Buddhists no longer cherish the relics and the Buddhists themselves began to lose faith in the Buddha as the ‘Enlightened One’. It is further prophesized that the Nagas will rise from the earth, through the water (their preferred form of entry to this realm), and take the relics and texts to Nagaloka. Then all of the knowledge of the Buddha and the Bodhisattvas will be lost until the Bodhisattva returns again (Snellgrove, 1987).

The intellectual community’s understanding of snakes differs from the mythology surrounding them. Snakes are believed to be immune to the bite of other snakes, it is unclear as to whether the snake produces anti-venom or is simply immune. The snakes that fall victim to the King Cobra’s (Ophiophagus hannah) hunger (as they feed primarily on snakes) are not immune to its venom while the King Cobra is immune to most snakes venom. Since Cobras do not constrict like Pythons or Boas, they will bite the snake preferably behind the neck area and suffocate it while injecting venom, which is less toxic than that of the Cobra (Naja naja). However this injection consists of about 6ccs, which is enough to kill an adult elephant.

The popularity of snakes is testimonial by its presence in various emblems and symbols. The serpent encircles the emblem of Ramkrishna Mission. It signifies that the vision of paramatma is obtained by the union of karma (as water), bhakti (as lotus), gyana (as rising sun) and yoga (as serpent). The emblem of many societies (Eg: The Pharmacological Society of India) has the snake as a part of their emblem.

The venom of the Indian Spectacle Cobra (Naja naja naja) contains a neurotoxin similar to that of the feared Black Widow spider. It quickly affects the nervous
system by inhibiting the neurological activity soon leading to paralysis and cardiac complications. Fortunately the Cobra commonly does not inject a fatal amount of venom. The venom of the Spectacle Cobra is being studied extensively at Tata Memorial Cancer Institute in Bombay and has been known to destroy cancer cells in mice.

Snakes carry a considerable amount of balance to local ecosystems, especially in a rodent’s paradise like India. Snakes have some amazing qualities, like lightening speed reflexes, the ability to go without food for months at a time when necessary and the complete independence since the time of birth.

**SOME COMMON INDIAN SNAKES**

**HARMLESS / NON-VENOMOUS SNAKES**

**Indian Rock Python** (*Python molurus*)

Average 3 to 5 meters in length, lay up to 100 eggs in June. Found throughout India, prefer arid scrub jungle and cool dense rain forest at 2000 ft above sea level or lower. Like all Boids (Boas and Pythons) they kill by constriction. Called Ajgar in Hindi and Moyal in Bengali (Fig.1).

**Common Sand Boa** (*Eryx conicus*)

Average 50cm to 1 meter in length, they are common throughout India on the plains and low hills but are rare or absent in most parts of Bengal and Assam. Like most snakes, they are primarily nocturnal and slightly resemble the saw-scaled Viper. They feed on rodents, lizards, birds and sometimes other snakes. Called Mati Ka Samp in Hindi, Bali Borha in Bengali.

**Red Sand Boa** (*Eryx johnii*)

Average 75 cm to 1 meter in length, the overall color is red, reddish-brown to black. They prefer to live in the drier parts of India and often live in rodent burrows. They give live birth to 6 to 8 young in June. They often feed on rodents and other snakes. The best defence of this animal is that its
tail looks just like its head. Predators become quick confused and disoriented and often leave the snake. Called Do Mu Samp in Hindi and Domundia in Oriya.

**Common Wolf Snake** (*Lycodon aulicus*)

Average 30 to 50 cm in length and commonly grey, brownish or black with 10 to 20 thin white bands. Smaller southern wolf snakes have somewhat translucent skin so the internal organs can be seen from the belly. There are over 10 species of wolf snakes and most are commonly seen in homes throughout India including the Andaman Islands. They are strictly nocturnal and are rarely seen during the day. Called Sakhara in Hindi and Ghorchiti in Bengali (Fig. 2).

**Banded Kukri** (*Oligodon arnensis*)

Average 35 cm to 50 cm and are typically reddish or grayish-brown with 10-20 black or dark brown bands. They are often found living in termite mounds or any other cave/burrow type hole. They feed on geckos, skinks and small mice. Although they don’t flourish in India they are often mistaken for kraits and immediately killed. Called Kukri Samp in Hindi and Udoy Kal in Bengali.

**Striped Keelback** (*Amphiesma stolata*)

Average 40 to 50 cm in length and are found throughout India. They are particularly fond of rice fields where they lay 10 to 12 eggs up to twice a year. Their diet is mostly frogs but they will also eat toads, lizards and rodents which are swallowed alive, they do not constrict. Called Hurwa in Hindi and Hele in Bengali.

**Trinket Snake** (*Elaphe helena*)

Average 70 cm to 1 meter and usually found on the plains. They are active during day and night except in the hot seasons when they spend most of their time in termite mounds or any other place they can find to keep cool. These snakes are often wrongly considered venomous, especially the green trinket of the Andaman Islands.
Fig 1: Indian Rock Python (*Python molurus*)

Fig 2: Common Wolf Snake (*Lycodon aulicus*)
Rat Snake (*Ptyas mucosus*)

Average 2 to 3 meters and are widely distributed throughout India. Rat Snakes vary in colour dramatically, from yellow to pitch black. The residents naturally see a bit of these snakes because they earned their name for their love of rats and where there are humans, there are rodents. The female lays 8 to 16 eggs between March and April. Along with cobras, rat snakes are the basis of a profitable, wide spread and cruel snake skin industry. Called Dhaman in Hindi.

Royal Snake (*Spalerosophis diadema*)

Average 1.5 to 2 meters in length and are only found in dry areas of Rajasthan, Punjab, Uttar Pradesh and Kashmir. Usual colouration is yellowish-brown with irregular black markings and a mostly black head. They are mostly active during the day and are extremely proficient climbers. Called Rajat bansi in Hindi.

Flying Snake (*Chrysopelea ornata*)

Average 1 to 1.5 meters in length and are usually black with white cross bands, this is a very vibrant looking snake. This snake is not considered venomous as they have no venom, but they do have rear fangs and a somewhat toxic saliva that is ineffective to humans. Snake charmer often try and pass this snake off as being ‘highly venomous.’ Called Kal Nagini in Bengali (Fig. 3).

Vine Snake (*Ahaetulla nasutus*)

Average one meter in length with a very pointed head. They are technically venomous as they are rear fanged which essentially means that they would have to gnaw to puncture skin. The most magnificent thing about this snake is its camouflage ability, in a patch of vines you will never find them. Called Hara Samp in Hindi, Laudoga in Bengali and Laudanka in Oriya (Fig. 4).

Bronzeback snake (*Dendrelaphis pictus*)

Average one meter in length, identified by its bronze head, black eye stripe and mask and a black lateral mark along the length of the body. It is a tree climber and feeds on small lizards and frogs. Called Betajra in Bengali (Fig 5).
Fig 3: Flying snake (*Chrysopelea ornata*)

Fig 4: Vine snake (*Ahaetulla nasutus*)
Fig 5: Bronzeback snake (*Dendrelaphis pictus*)

Fig 6: Checkered Keel (*Xenodrophis piscator*)
Checkered Keelback (*Xenodrophis piscator*)

Common snake with an average length of one meter. They haunt watery areas like wells, tanks and canals. They feed on frogs. Called Dhora Saap in Bengali (Fig 6).

VENOMOUS SNAKES

Common Krait (*Bungarus caeruleus*)

Average one meter in length and are usually jet black with white cross lines. The common krait is one of 6 species found in India but the most common. Kraits are also considered one of the 4 most dangerous snakes in India. The common krait prefers moist soil so they are often found around the coastal areas, but kraits can be found throughout India. Called Maneer in Hindi, Kalaj in Bengali and Chitti in Oriya (Fig 7).

Indian Spectacle Cobra (*Naja naja naja*)

By far, the most well known of Indian snakes averaging 1 to 1.5 meters in length. They are found throughout India and are considered one of the four most dangerous snakes in India. They are commonly found in rice fields with a large rodent population. A common cause of cobra bites are people working in rice fields and unknowingly stepping on a cobra. They lay 12 to 30 eggs once a year and the female will usually stay with her eggs for the entire 60 days gestation period. The venom of the Cobras are neurotoxins which quickly causes respiratory and cardiac complications, fortunately for humans usually less than fatal amounts are injected. Called Nag Samp in Hindi, Gokhro in Bengali and Gokhara in Oriya (Fig 8).

Indian Monoocled Cobra (*Naja Naja kaouthia*)

This snake is virtually identical to the *Naja naja naja*. The *Naja kaouthia* usually is shorter by 10 cm on average, they lay a few less eggs. Both will choose flight over fight any time they feel it is an option. Called Nag Samp in Hindi and Keoute in Bengali (Fig. 9).
Fig 7: Common krait (*Bungarus caeruleus*)

Fig 8: Indian Spectacled Cobra (*Naja naja naja*)
King Cobra (*Ophiophagus hannah*)

Not extremely common in India they are confined to the dense forests of Northern India. They are the largest venomous snake known today and have an extremely inaccurate reputation for being aggressive. They are also the only known nesting snake, they will actually construct nests for their eggs. The nearest antivenom for King Cobras is in Thailand. Called Naga Raja in Hindi, Sankhachoor in Bengali and Ahiraja in Oriya.

Nosed Sea Snake (*Enhydrina schistosa*)

Average 60 to 90 cm in length, they are rather short and stalky. They have a flat paddle-like tail and the overall color is grey with a white under belly. They are found on the coastal regions of the main land, Sri Lanka and most islands. They are the most common of the 20 species of sea snakes found in India. They can dive to 100 meter and stay submerged for up to 5 hours at a time. Most fishermen have little fear of these snakes, like most sea snakes they rarely bite and are responsible of an extremely small amount of bites each year. This snake is by no means harmless, its venom is rated 8 times more toxic than that of *Naja naja naja* and antivenom is only found in Australia and Japan. Called Dariya Samp in Hindi.

Russell’s Viper (*Vipera russelli*)

Average 1 to 1.5 meters in length. They are usually shades of brown with very rough scales. They resemble the harmless common sand boas. They are considered one of the four most dangerous snakes in India. Typically if large amounts of antivenom is not administered within an hour of a bite, the victim will die. The majority of bites occur on plantations when workers step on them or startle them. Again, this snake would choose flight over fight any day. Called Gonus in Hindi and Chandra Borha in Bengali (Fig 10).

Saw-Scaled Viper (*Echis carinatus*)

Average 30 cm in south India and 50 cm in Northern India. They are primarily nocturnal and are brown, grayish with zigzag patterns. Female bear 6 to 8 live young in fall. They eat mice, lizards, frogs, scorpions and other anthropods. In many areas this snake is the largest cause of bites. Called Phoorsa in Hindi and Dhuli Naga in Oriya.
Fig 9: Indian Monocled Cobra (*Naja naja kaouthia*)

Fig 10: Russell's Viper (*Vipera russelli*)
Banded Krait (*Bungarus fasciatus*)

Average 90cm or more, with smooth and shiny scales, wide bright yellow and black bands on its back. They have a prominent backbone, blunt tail and rounded head. They are found in Assam, Bengal, Bihar, Orissa and parts of Madhya Pradesh. Called Shakhamuthi in Bengali (Fig. 11).

**EVOLUTION OF SNAKE**

The first snake-like animal appeared on the face of earth, about 100 million years ago, during Cretaceous period. Snakes, like all living things, are the product of the process of evolution, which allows species to change over time in response to environmental factors to produce entirely new species.

The oldest group of very ancient reptiles known as the *Cotylosaurs*, or 'stem reptiles', are believed by paleontologists to be ancestral to all of the reptile families alive today. They appeared during the time that immediately preceded the rise of the dinosaurs. Thus, they are believed to be the evolutionary cousins of dinosaurs.

One of the earliest snakes to appear in the fossil record has been given the scientific name *Lapparentophis defrenni*, found in the Saharan Desert dating about 130 million years ago. Another very early snake, which lived about 100 million years ago, called *Simoliophis* has been found in marine deposits in North Africa and Europe. The most complete skeleton of a fossil snake named *Dinilysia patagonica* was found in Upper Cretaceous rocks in Argentina. It shares many anatomical characteristics with the modern boas and pythons, which are usually considered to be the most primitive of the living snakes. Another fossil snake, *Gigantophis*, that was found in Egypt, had an estimated length of over fifty feet, and is the largest of all the known snakes. It was also related to the modern boids. One of the most interesting snake fossils is the extinct boid *Paleryx*, found in Germany. Fossils of this ancient snake have been found which still contain the impressions of the scaled skin. Based on these fossil finds, as well as on anatomical study of modern reptiles, scientists have concluded that the snakes probably evolved from a family of lizards during the time of the dinosaurs.
Fig 11: Banded Kukri (*Bungarus fasciatus*)
The first of the modern terrestrial snakes to appear seem to have been relatives of the living boids, or boas and pythons. After the dinosaurs disappeared, the boids were the dominant snake family on earth, and became widespread and very diverse. About 36 million years ago, however, a group of smaller, faster snakes appeared. These were the colubrids, who were unable to outcompete the boids and remained a small group of snakes until about 20 million years ago, when the continental plates began to reach their present positions. The boids disappeared from many areas and were greatly reduced in number and diversity. The colubrids quickly moved and soon dominated the snake world. Today, the colubrids make up over two-thirds of all the living species of snakes.

About 15 million years ago, snakes began appearing which had a number of greatly enlarged teeth at the rear of their jaw. Today, such snakes are referred to as *Opisthoglyphs* or ‘rear-fanged’ snakes. Shortly after the *Opisthoglyphs* appeared, another group of snakes developed a more refined venom apparatus. These snakes are known as *Proteroglyphs*, and are classified as the Elapids having short fixed fangs in the front of the mouth, which can be used to bite and strike at enemies as well as food. Living descendants of the Elapids include the cobras and the sea snakes.

By about 10 million years ago, the most highly specialized of the snakes appeared in the fossil record—the *Solenoglyphs*, commonly known as vipers having fangs much larger than in the Elapids. A short time after the vipers appeared, a group known as the pit vipers developed a number of heat-sensitive pits on the front of the face, which they used for finding their warm-blooded prey at night. Finally, just a few million years ago, a group of pit vipers developed a structure at the end of their tail, made up of interlocking pieces of unshed skin, which could be loudly rattled and used as a warning device against predators. The rattlesnakes are generally thought to be the most specialized of all the living snakes.

Newer techniques using molecular biology may give us a more complete picture of snake evolution. The study of snakes using DNA techniques is still in its infancy, but has already revealed a few surprises. Preliminary results
indicate that the vipers are not, as was formerly thought, the most recent of 
the snakes, but instead diverged from the ancestral boid stock before both the 
elapids and the colubrids. Much work remains to be done on the evolution 
of snakes.

STRUCTURE OF A SNAKE

Almost all the types of snakes have an elongated body, divided into a 
head, body and tail. They do not have any external appendages or limbs. 
Exceptions are that in Typhlops species which have a few internal rudiments 
and some are found in Python. The entire body is covered with scales forming 
a distinctive characteristic. Snakes moult or shed the old skin at regular intervals: 
They have eyes without eyelids and have large pupils. External ear is absent. 
Their lower jaw is not a single bone and is connected in front by an elastic 
ligament. Snakes have a forked tongue which comes out through the opening 
in the lower jaw. The jaws have curved pointed teeth. Venomous snakes possess 
a form of modified teeth called fangs. The length of the snake can vary between 
the smallest 73mm and the longest 10 metres.

CLASSIFICATION OF SNAKES

Snakes belong to the class Reptilia and are classified under the order 
Ophidia. Snakes are divided into 14 families:

1. Acrochordia 
2. Anilidae
3. Anomalipidae 
4. Boidae
5. Bolyeridae 
6. Leptotyphlophilidae
7. Typhlophilidae 
8. Uropelldae
9. Xenopeltidae 
10. Colubridae
11. Crotolidae 
12. Elapidae
13. Hydrophiidae 
14. Viperidae

The last 5 families comprise of all the venomous snakes of the world. (Bjarnason 
& Fox, 1988-89).

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The family **Colubridae** comprises of the largest family with two thirds of all snakes belonging to this group. They are mostly nonvenomous and have no fangs. Exception is that of the African boomslang *Dipholidus typus* which is venomous.

The **Crotolidae** family are commonly known as pit vipers due to the presence of temperature sensitive organs located in a pair of pits on both sides of the head between the nostril and the eye. There are two pit vipers found in India. They are the Himalayan pit viper (*Ancistrodon himalayanus*) and Bamboo pit viper (*Trimeresurus gramineus*).

Sea snakes belong to the group **Hydrophidae**. Some of them are extremely poisonous. They have short, immobile fangs and flat rudder-like tail. The most venomous snake in the world *Hydrophis belcheri* is a sea snake. Its venom is myotoxic which is hundred times as toxic as that of Australian taipan (*Oxyuranus scutellatus*) whose bite can kill a man in minutes. *Hydrophis caerulesceus*, a sea snake is found all along coastal India. Fishermen are its major victims.

The family **Elapidae** is the second largest group of venomous snakes. The Cobras, Kraits, Mambas, Coral are its members. They have short non-mobile fangs (3-5mm) in adults. They are widely distributed in the tropical region. India harbours the largest venomous land snake in the world *Ophiophagus hannah*, which is an elapid.

The members of the **Viperidae** family are commonly called Vipers and some of them are deadly. The Saw-scaled or Carpet Viper (*Echis carinatus*) kills more people in the world than any other snake. *Vipera russellii* is another well known species of this family which is abundant in South Asia including India.
DISTRIBUTION OF SNAKES

Snakes are widely distributed in all the geographical regions between Arctic and Antartic Circles. They are densely populated in the tropical forests. Venomous snakes are distributed from sea level (*Agkistrodon*) to altitudes of more than 5000m above sea level (*Gloydius himalayanus*) (Warrell, 1996). Sea snakes are found between altitudes 30°N and 30°S. They are also present in extreme north as Siberia (*Pelamis platurus*), snakes are found in estuaries, rivers and freshwater lakes eg: *Enhydrina schistosa* in Ton Ley Sap, Cambodia (Warrell, 1994). Snakes are not found in areas like the two Poles, Islands of western Mediterrannean Sea (including Crete), the Atlantic and Caribbean Sea, Madagascar, New Caledonia, New Zealand, Hawaii, few islands of Pacific Ocean, North America north of latitude 51°N, Newfoundland, Nova Scotia, Ireland, Iceland and Chile (Klemmer, 1963).

SOME FACTS ABOUT SNAKES

Longest snake

◆ **Non venomous**: The reticulated Python (*Python reticularis*) is about 20 ft 6" long. The longest snake recorded was a 10 m. (32 ft 10 in) long reticulated python killed in 1912 on the island of Celebes.

◆ **Venomous**: *Ophiophagus hannah* of Southeast Asia is about 12-15 ft in length. In the 1930s, one specimen grew to a length of 5.71 m. (18 ft 9 in) at London Zoo.

Shortest snakes

◆ **Non venomous**: The rare thread snake, *Leptotyphlops bilineata* found only in the island of Martinique in St Lucia, West Indies, measuring about 108 mm.

◆ **Venomous**: The Namaque dwarf adder (*Bitis Schneider*) of Namibia has an average length of 8 inches.

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Heaviest snakes

- **Non venomous**: The *Eunectus murinus* of tropical South America and Trinidad is twice as heavy as *Python reticularis* of the same length (18-20 ft) weighing 227 kg.

- **Venomous**: Diamondback rattlesnake (*Crotalus diamasteus*) of the United States have the average weight of 5-7 kg, the heaviest on record being 15 kg. The West African gaboon viper (*Bitis gabonica*) of the tropical rain forest is probably bulkier, about 11.34 kg.

Most venomous

- The sea snake *Hydrophis belcheri* is hundred times as toxic as the Australian taipan (*Oxyuramus scutellatus*) whose bite kills a man in minutes. The most venomous land snake is the Australian *Parademonisia microlepidotus*, whose venom is nine times more toxic than the tiger snake (*Notechis sculatus*) of the same region.

Most fatal

The most fatal snake is the Saw scaled viper (*Echis carinatus*) found between West Africa to India. Its bite can kill more people than any other species in the world.

Longest fangs

Gaboon viper (*Bitis gabonica*) has the longest fangs in the world (50mm).

Oldest snake

The highest reliable age recorded so far is 40 years 3 months 14 days for a common snake (*Boa constrictor constrictor*) named Popeye, who died in Philadelphia zoo in 1977.

Fastest snake

The black mamba (*Dendroaspis polylepis*) found in eastern Africa is probably the fastest land snake. It attains a speed of 16-19 km/hr in short bursts.

Rarest snake

*Liophis arnatus* is considered to be the rarest snake on earth. It is found only in Maria island of St.Lucia, West Indies. The round Island Boa (*Bolyeria multicarinata*) is known from only two specimens and has probably become extinct in early 1980.
HABITS OF SNAKES

Habitats: Although temperature limits their activity and numbers in countries with cool climates, in tropical regions snakes have moved into many habitats. There are totally aquatic snakes, including sea snakes, snakes that live their entire lives beneath the ground and others that spend much of their time in the upper branches of huge forest trees. Each species is well adapted to its habitat, in shape, colour and behaviour, leading to a much wider variety of size, shape and colour than is generally realised.

Feeding: Snakes generally feed once after a long time. The fat is stored and is utilized during hibernation. Large snakes feed on mice, rats and frogs. The water snakes catch the fish and frogs and sea snakes feed on fish and eels. Kraits and Cobras devour other small snakes as their food. The small snakes feed on lizard, scorpions and earthworms. Snakes are mostly carnivorous except some egg eating colubrine snakes (the African genus Dasypeltis and Indian Elachistodon westermanaii). Venomous snakes immobilize their prey with the venom while large non venomous snakes squeeze their prey to death by powerful coils (Whitakar, 1978).

Mating: Snakes mate once a year with the same species. Each species have a different mating season. Snakes have a distinct reproductive system. The male has a paired hemipenis in tail. Both the sexes have musk gland at the base of the tail. It produces secretion to attract the opposite sex (Whitakar, 1978).

Reproduction and hatching: Reproduction is quite varied among snake species. Most lay eggs, which have a pliable shell through which water is absorbed during the development of the embryo. These eggs are laid in a secluded place with a stable temperature and some moisture. Snake eggs can take up to three months to hatch and the female that lays them usually plays no part in their future development (Fig 12).

Other species retain their eggs inside their bodies, without shells, until they are at the point of hatching. Their young are fully formed when born. Giving birth to live young is an adaptation to cold environments by retaining the eggs inside their bodies the females can help the incubation along by basking and keeping their bodies warm. Snakes that lay eggs must rely on the weather.
to provide enough heat for incubation. Other groups of snakes are live-bearing for other reasons: aquatic snakes, including most of the sea snakes, are unable to lay eggs because they rarely, if ever, come onto the land. Similarly, tree-dwelling species tend to be live-bearing to avoid having to move down to ground level.

A single species of snake, the Brahminy Blind Snake is parthenogenic. This means that there are only females of this species. Once they reach breeding size they start to lay eggs without needing to mate with a male. All of the eggs hatch into females.

Young snakes are able to fend for themselves as soon as they hatch or are born. They usually shed their skin within a few days and then they search for a suitable place to live and hunt for food. Growth rates vary, but small species may be large enough to breed within one year, medium-sized snakes take two to four years to reach maturity, and large snakes, such as boas and pythons, take at least four years sometimes longer.

**Defence**: The defence of snakes differ between species. For example, the Russell’s Viper and Python hiss by forcibly inhaling and exhaling. The Cobras make a dramatic display of raising their hood by expanding their ribs. While some other snakes just remain still and do not attract attention of the enemy. However the enemies dread the snake because of their ill-famous defence, the venom. Very few snakes are fortunate to have this defence mechanism.

Snakes have many enemies and they have several means of preventing themselves from being killed or eaten. The most effective and common method is to avoid detection. Many are well camouflaged so that they blend into their natural surroundings: tree snakes are green and brown, desert snakes may be yellow or light brown, etc. Others have very intricate markings consisting of blotches, bands or stripes and these are intended to confuse predators by disguising the outline of the snake. Yet other species are brightly coloured, with contrasting bands of red, yellow, white, black and so on. This is to warn or frighten potential enemies, but it may also create an optical illusion. When the snake moves and the bands flicker quickly past, the predator may be confused as to which direction the snake is travelling.
If a snake fails to avoid detection, or if its warning colours do not deter an enemy, it may resort to other means of defence. Many species bite, even though only a few are venomous, and a number of them hiss and puff up their bodies in order to appear larger and fierce than they are. Others form themselves into a ball, with their heads safely tucked away among the coils and they may raise their tail and wave it about as if it is their head. A few play dead by turning over onto their backs and allowing their tongue to hang out of their mouths, this is often accompanied by the secretion of a foul-smelling substance from glands at the base of their tail.

The rattle snake have a unique way of warning their enemies, their rattle is formed from parts of old shed skins, which are trapped by a constriction at the end of their tail. Each time the snake sheds its skin a new segment added and the rattle gets longer (although the end of the rattle may get broken off from time to time). By vibrating its tail, the rattle snake makes a loud buzzy noise, warning predators that it is dangerous and also alerting large animals, such as a cattle, to its presence.

Many snakes are becoming increasingly rare. Although many reasons contribute to their decline, habitat destruction is the most important one. Numerous habitats are shrinking, through the activities of agriculture, forestry, urbanisation and road building. Some species are protected and collecting or disturbing them is illegal. Unfortunately, these measures only protect individual snakes, thousands are killed by road traffic each year and hundreds of thousands die because the places where they live, are destroyed or altered. Snakes have an important role in the web of life. Many do a useful job of controlling the number of rodents and other pests and they all have a right to exist.

Senses: Snakes do not have eardrums and openings. Hence they can be called deaf. Yet they can feel the vibration of the airborne sound via the lungs. Snakes have a sharp visual capacity but they do not readily react with objects which are stationary. Most snakes have the sense of smell. They also feel pain and can suffer from changes in temperature and humidity.
**Locomotion** : Most of the snakes can climb and almost all can swim. Some can even glide in air. Many tree snakes like bronzebacks have notched belly scales and thin strong bodies which help them to climb trees. The sea snakes use paddle tails for propulsions and can hold their breath for 5 hours. Earthsnakes like sand boas are adapted to burrow into the soil by having strong shovel or pointed shaped heads.

**THE VENOM APPARATUS**

The components of venom apparatus are a pair of venom glands which produces and stores the venom and the modified teeth that attaches to the glands, called the fangs which delivers the venom.

**The venom glands** : The occurrence of the oral gland is probably due to their adaptation to the swallowing of large prey (Gans, 1961). There are three types of venom glands Elapidae, Atractaspis (Mole Viper), Viperidae (Bobeau, 1936). The elapidae gland as an enclosure in a tough capsule of connective tissue more compactly built than that of the Viperid snake. It can be divided as a posterior main gland and an anterior secretory duct with an accessory mucus gland. The main gland is composed of many contagious tubules which may be simple or compound. They run in a postero-anterior direction and converge into the centre of the gland. Finally, they open into a small lumen. The venom glands of the viper have four regions - the main gland, the primary duct, the accessory gland and the secondary duct that leads to fang sheath.

**Fangs** : Fangs help the snake to deliver the venom that is stored in the glands. They are specialised teeth and are larger than the other teeth (Fig. 13). The fangs have a deep groove that run along its rostral edge. A tube like canal penetrates the fangs from its base almost to the tip and this also opens on its rostral side. The large fangs of vipers and crotalid snakes can be laid backwards by a turning movement of maxillary bone, into a sheath of mucous membrane of the mouth. The elapid (spitting Cobra) can spit venom with the help of their lungs. The gaboon viper (Bitis gabonica) have the largest fangs (50mm).
Fig 12: Newly hatched *Naja kaouthia* from eggs

Fig 13: Fang of snake (*Vipera russellii*)
Section 2

SNAKE VENOM AND ITS CONSTITUENTS
Venom can be defined as a toxic secretion produced by specialized tissue or gland (venom gland). These glands can be connected to the structure which delivers it (fangs, stings). The venom accumulates in the lumen of the gland and is extruded during a bite. Venom is a complex mixture of mainly proteins and peptides of low molecular weight, nucleotides and metal ions.

Snake venom is the most widely studied animal toxin. Its composition differs from one snake family to another (elapidae, viperidae, crotolidae and hydrophidae) and even within the same family. For example, the two species of *Naja* and *Hemachatu* of the 46 species of elapidae family, contain cardiotoxins. Neurotoxins are present in most of the venoms but they still differ depending on the types of membrane receptor or channels on which they act.

**Physical properties of snake venom**

Snake venom is colourless or yellowish, feebly acidic fluid. It’s specific gravity is 1.46. The lyophilized (dried) form can retain its toxicity and biological properties for many years. Russell’s viper venom is usually white or yellow in colour and is soluble in water, when in high concentration. Cobra venom is slightly viscous in nature and it becomes turbid if exposed to sun. Solubility of the elapid venom is greater than the viperid venom. Solubility of all the venoms is increased in physiological saline (Fig. 14).

**Chemical composition of snake venom**

Chemical composition of snake venom is very complex. Major part of the venom is protein or peptides and remaining portion is composed of inorganic components, like nitrogen, phosphorous, chloride ions and certain metals. Chemical composition of three major venoms is given in the next page.
Fig 14: Lyophilized snake venom
<table>
<thead>
<tr>
<th>Constituents</th>
<th>Naja naja</th>
<th>Vipera russellii</th>
<th>Crotalus terrificus terrificus</th>
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<tr>
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<td>15.8%</td>
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<tr>
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<td>—</td>
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<td>GLOBULIN</td>
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<td>—</td>
</tr>
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<td>12.02/100mg</td>
<td>14.0/100mg</td>
</tr>
<tr>
<td>TOTAL INORGANIC</td>
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<td>5.71/100mg</td>
<td>5.46/100mg</td>
</tr>
<tr>
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<td>0.19%</td>
</tr>
<tr>
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<td>—</td>
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<td>1.86%</td>
</tr>
<tr>
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<td>0.22%</td>
</tr>
<tr>
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</tr>
<tr>
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<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MAGNESIUM</td>
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<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Neurotoxin**

Neurotoxins are the most toxic components of snake venom. It interferes with the nerve muscle physiology specially the neuromuscular junction. Based on the site of action, they are classically divided into presynaptic and postsynaptic neurotoxins. The presynaptic neurotoxin inhibits the release of acetylcholine and the lethality therefore is very high. The postsynaptic neurotoxin binds to the nicotinic acetylcholine receptors preventing the depolarizing action of the acetylcholine. These toxins are usually referred to as curaremimetic, curariform or curare-like toxins. Snakes belonging to the families *Elapidae* (Cobras, Krait, Mambas, Tiger snakes, Coral snakes) and *Hydrophidae* (sea snakes) have typical neurotoxic venom with a high neurotoxic content.

**Post synaptic neurotoxins**

Post synaptic neurotoxins are also called α-neurotoxins or curare-mimetic toxins, because their action is similar to that of an alkaloid poison, d-tubocurarine (curare). They bind to the nicotinic acetylcholine receptor on the post synaptic membrane of the neuromuscular junction. This prevents the binding of acetylcholine to its receptor. The onset of the action of snake venom is slow.
but its duration is longer as compared to curare and it is specific to nicotinic cholinergic receptor (Hassan, 1962). In other words, the venom toxins are bound much more strongly to the receptors than curare thus making them more potent. These toxins are found in Proteroglyphous snakes (the fangs of these snakes are fixed to the front jaw, unlike the Viperidae) of the Elapidae and Hydrophidae families.

The earliest report of the chemical nature of cobra venom was provided by Brunton and Fayrer (1873). They showed that the cobra venom is both neurotoxic and cardiotoxic. Death of a victim occurs due to respiratory paralysis resulting from its action on the nerve endings. They also reported that cobra venom is heat stable and the toxins are protein in nature with a low molecular weight.

Studies with the neurotoxin were conducted by many workers starting with isolation of the toxin (Kaiser and Michael, 1958; Porath and Flodin, 1959; Devi, 1968; Ghosh and Chowdhury, 1968) by fractional precipitation with various salts, gel electrophoresis, adsorption chromatography and gel filtration. Gradually the purification process have been simplified. The components are soluble in aqueous buffer, centrifuged to remove small amounts of insoluble inactive material, the samples are purified by chromatography. Complete structure can be determined by amino acid sequencing.

More detailed studies have been done in recent times. It is now concluded that the postsynaptic neurotoxin also called α-neurotoxin, is a flat molecule. It can be divided into two classes, the short and the long neurotoxin. The short neurotoxin are 60-62 residues long containing 4 disulphide bridges. They are of two types: neurotoxins ‘a’ and ‘b’ isolated from the Phillipines sea snake, Laticauda semifasciata (Tsermoglou and Petsko, 1976,1977). They were found to be identical to erabutoxins ‘a’ and ‘b’ from the Japanese L. samifasciata (Low et al., 1976; Corfield et al., 1989). The toxin ‘b’ was found to differ from ‘a’ by only one residue. The other toxin, the long neurotoxin have 70-74 amino acids and 5 disulphide bridges (Endo and Tamiya, 1987, 1991). The short neurotoxins are erabutoxins from L. semifasciata (Inagaki et al., 1980), neurotoxins I and II from Naja mossambica mossambica (Lauterwein et al., 1978).
and cobra toxin from *Naja naja atra* (Endo et al., 1979). The long neurotoxins, α-cobratoxins from *Naja naja siamensis* (Walkinshaw, 1980) and α-bungarotoxin from *Bungarus multicinctus* (Agard and Stroud, 1982) have been studied in details. The long neurotoxins are toxin LS III from *L. semifasciata* (Inagaki et al., 1981), α-bungarotoxin from *B. multicinctus* (Inagaki et al., 1985; Hider et al., 1982a) toxin B from *Naja naja* (Endo et al., 1981) and a cobratoxin from *Naja naja siamensis* (Hider et al., 1982a). Recently, a weak neurotoxin designated as WTX, was isolated from the *Naja kaouthia* venom. The sequence of this neurotoxin revealed that WTX is the first case of tryptophan containing weak neurotoxin (Utkin et al., 2001).

The mechanism of action of the neurotoxins is still not clear. The interaction with the nicotinic acetylcholine receptor of the neuromuscular junction with the toxins are yet to be proved. One of the possible mechanism of action is by its ‘toxic site’ present in the venom or extensive surface contact with the acetylcholine receptor. The pH is crucial for the toxic activity of the venom. The neurotoxin is stored at low pH in the venom gland but acts at physiological pH when injected. There is no evidence regarding pH dependent conformational change (Hider et al., 1982b).

**Presynaptic neurotoxin**

The presynaptic neurotoxin, as the name suggests, acts on the presynaptic site of the neuromuscular junction. These toxins are also called β-neurotoxin. Acetylcholine release is inhibited by them. The lethality of the presynaptic toxin is much more than the postsynaptic neurotoxin. Presynaptic neurotoxins are found both in elapid and viperid snakes. They act by interfering the release of acetylcholine or other neurotransmitter. It is due to the inhibition of a voltage sensitive K⁺ channel. Their structures are similar to that of Phospholipase A₁. The neurotoxic and non-neurotoxic snake venom phospholipases are homologous to phospholipases from mammalian pancreas. Besides being neurotoxic, they can also be myotoxic, cardiotoxic, haemolytic and may affect platelet aggregation.

Snake venom **Phospholipases** form a vast and diversified family. Neurotoxic phospholipases include notexin (Australian Tiger snake) taipoxin (*Oxyuranus* (28))
scutallatus scutallatus) and \( \beta \)-bungarotoxin (B. multicinctus) all from Elapid snakes and caudoxin (Bitis caudalis) and crototoxin (Crotalus durissus terrificus) from viperid snakes (Kini and Iwanaga, 1986). All of the above toxins have the \( \text{PLA}_2 \) like structure, with exception of \( \beta \)-bungarotoxin. Myotoxin, a Lys49 phospholipase \( \text{A}_2 \) homologue was isolated from the snake venom of Bothrops moojeni (Caisacca).

Taipoxin is a ternary complex of three non identical subunits which act synergistically. This confers extreme toxicity to the toxin (Karlson, 1979). The venom of Australian taipan snake (Oxyuranus s. scutallatus) is extremely potent due to the presence of taipoxin. The two protein subunits of taipoxin were found to be mitogenic having neurotrophic activity on PC12 cells in culture similar to nerve growth factor (Lipps, 2000). Crotoxin is made up of two subunits, both of them homologous with \( \text{PLA}_2 \), even though the second subunit crotapotin, is cleaved into three polypeptide units (Bon, 1982). Vipoxin and \( \beta \)-RTX are isolated from the venom of Vipera russelii, also belong to the \( \text{PLA}_2 \) family (Bevan and Hiestando, 1983; Freedman and Synder, 1981). The vipoxin and \( \beta \)-RTX toxins act on the non cholinergic receptors. They were found to inhibit biogenic amine receptors (adrenergic, dopaminergic and serotonergic receptors). Myotoxin, was purified from the snake venom of Bothrops moojeni by ion exchange chromatography. It has 121 amino acids and a molecular weight of 13,669. It is homologous to Lys 49 \( \text{PLA}_2 \). It shows local myotoxic and edema inducing activity (Soares et al., 2000). Myotoxicity and edema has also been induced by an acidic phospholipase \( \text{A}_2 \) denoted LM-\( \text{PLA}_2 \), isolated from Lachesis muta snake venom (Fuly et al., 2000). The \( \text{PLA}_2 \) and crotapotin subunits of crotoxin from Crotalus durissus cascavella venom were purified by a combination of high-performance liquid chromatography (HPLC). The amino acid composition of the \( \text{PLA}_2 \) showed the presence of 14 half-cysteines and a high content of basic residues (Lys, Arg, His), whereas the crotapotins were rich in hydrophobic, negatively charged residues and half-cysteines. Crotapotin (F3) and heparin inhibited the catalytic activity of the \( \text{PLA}_2 \) by acting as allosteric inhibitors (Beghini et al., 2000).

Dendrotoxins have been isolated and sequenced from the venom of mambas. Protease, trypsin or chymotrypsin inhibitors have been found in the
venom of cobras or vipers (Harvey and Anderson, 1991). Dendrotoxins facilitates the release of acetylcholine and of a variety of other neurotransmitters at peripheral and central synapses, an effect opposite to that of β-bungarotoxin which irreversibly blocks transmitter release (Chapell and Rosenberg, 1992). However, they can inhibit β-bungarotoxin, indicating that these toxins share a common binding site (Black and Dolly, 1986).

**Other neurotoxins**

Besides presynaptic and postsynaptic toxins, there are other neurotoxins which act in a different manner.

**Toxins acting on other cholinergic receptors**

These toxins are postsynaptic neuronal toxins that block nicotinic acetylcholine receptors of the peripheral nervous system, rather than the neuromuscular junction (Chiappinelli, 1991). This toxin exhibits a considerable sequence homology with α-bungarotoxin, but with a few differences. A different type of acetylcholine receptor in the central and peripheral nervous system is called muscarinic because these receptors are not sensitive to nicotine but to muscarine (mushroom toxin). In recent times, two toxins have been characterized from the venoms of green mambas (Adem et al., 1988) which affect these receptors.

**Fasciculins**

The toxin fasciculins is found in only one snake species *Dendroaspis* (mamba snakes). They inhibit acetylcholinesterase. This enzyme hydrolyzes the acetylcholine molecules released into synaptic space of the neuromuscular junction and thus lead to termination of the nerve impulse. Fasciculins act in synergy with dendrotoxins, which stimulates the release of acetylcholine. In contrast to neurotoxins which block transmission, fasciculins and dendrotoxins increase it beyond control, thus causing a permanent blockade of muscle contraction.

Crystalline structure of two fasciculins have been determined by X-Ray diffraction (Le Du, 1992). These toxins have 61 residues, 4 disulphide bridges.
and the residues similar to short neurotoxins and cardiotoxins. The structure is very similar to the cardiotoxin.

**Cardiotoxins**

The word 'cardiotoxin' is derived from the greek word 'cardia' meaning 'heart' and 'toxin' meaning poison. The cardiotoxins found in the venom of cobras (\textit{Naja} and \textit{Hemichatus}) are primarily basic proteins. They are less toxic than neurotoxins.

The first observation of the failure of blood circulation after intravenous injection of cobra venom was made by Chopra and Ishwariah (1931). Epstein (1930) showed that a significant change in the rhythm and conduction of heart on injection of the same venom. The cardiotoxic factor was also isolated from \textit{Naja naja} venom which blocks the heart in the systolic phase (Sarkar, 1947). Different cardiotoxins were isolated from different snake venoms. Toxin V\textsubscript{11} from \textit{N. mossambica mossambica} (Rees et al., 1990), Toxin Y from \textit{N. nigricollis} (Menez et al., 1992) and from \textit{Naja naja atra} (Chen et al., 1991).

Cardiotoxin is less lethal than neurotoxin. It blocks heart by depolarizing muscular membranes, leading to contraction of smooth and skeletal muscle. It also depolarizes nerve cells, causes cytosis and hemolysis and prevents platelet aggregation (Harvey, 1985). The cardiotoxin isolated from \textit{Naja naja atra} causes contraction followed by paralysis of chick biventer cervices, frog sartorius muscle and rat phrenic nerve diaphragm preparation. There was irreversible blockade of action of the muscle cell membrane. Cardiotoxin causes systolic arrest in isolated frog heart and rat atrium and contracted isolated guinea pig ileum. The toxin produced a fall of blood pressure in anaesthetized cats accompanied by various electrocardiogram changes (Lee et al., 1968).

It has been reported that the cardiotoxin affects a membrane calcium binding site and induces contracture by releasing the membrane calcium rather than increasing the sodium permeability of the muscle membrane (Lin et al., 1976).

Cardiotoxin isolated from \textit{Naja naja atra}, potentiates platelet aggregation induced by ADP, thrombin and PLA\textsubscript{2} of venom. Malondialdehyde formation
caused by ADP, thrombin and MILA₂ was increased in the presence of cardiotoxin. Both the platelet aggregation potentiation and increase in the malondialdehyde formation were blocked by indomethacin or Ca⁺⁺ (5mM or 0.05mM). Cardiotoxin did not potentiate thrombin-induced aggregation of p-bromophenyle bromide modifies platelets. Cardiotoxin also increased thrombaxane B₂ formation induced by thrombin or collagen but did not affect arachidonic acid (Fletcher and Jiang, 1993).

Cardiotoxin possesses the haemolytic activities which are also potentiated by PLA₂ (Lee et al., 1972). The cardiotoxin isolated from Naja naja siamensis venom produced irreversible blockade of concentration in embryonic heart both in in vitro and and in vivo (Arms and McPheeters, 1975). It was also noted that early embryonic heart is less susceptible than mature heart to the inhibitory effect of cardiotoxin.

Two cardiotoxins isolated from Bungarus fasciatus venom possessed the different actions with that of cardiotoxin from Naja naja atra (Lin et al., 1972). The toxin possessed contracture producing activity on chick biventer cervices, produced local irritation of rabbit eye conjunctiva and showed a light depression on isolated frog heart and rat atrium. It did not produce hemolysis.

Cardiotoxins are basic proteins and are most abundantly found in the cobra species. It is stable in acidic pH and its molecular weight varies from 6000-7000, with only one exception. A cardiotoxin isolated from Crotalus scutellatus has a molecular weight of 22,000. From the structural point of view, 3D structure confirms that cardiotoxins are very similar to the neurotoxins. Cardiotoxins consist of 60-62 amino acids, reticulated by 4 disulphide bridges. More than 60 primary sequences are available. Cardiotoxins are rich in hydrophobic and basic amino acids. Cardiotoxins are difficult to crystallize. It is because the hydrophobic residues of the cardiotoxin belong to different strands of polypeptide chain and remain in a cluster. Due to the external hydrophobic region, there is constraint on crystal packing, because this region remains hidden from the solvent. The hydrophobic and the basic proteins are functionally important. It was found that cardiotoxins attack and disrupt membranes and also penetrate acidic phospholipids monolayers (Bougis et al., 1981).
Cardiotoxins essentially depolarizes skeletal muscles. This activates the voltage dependant Ca\(^{2+}\) channels with subsequent release of Ca\(^{2+}\) from the sarcoplasmic reticulum leading to muscle contraction. It was also shown that contraction can occur even in low sodium media (Lin et al., 1976) which suggests that membrane depolarization is not an absolute prerequisite. It was also proved that cardiotoxin induces muscle contraction by activation of an endogenous Ca\(^{2+}\) channel (Dufton and Hider, 1983). The mechanism of depolarization is still not clear but suggested that it was due to the formation of pores which would allow the free passage of ion (Harvey et al., 1982). It was also known that cardiotoxin interact with negatively charged phospholipids in natural and synthetic membranes (Vincent et al., 1978). The affinity of cardiotoxins for negative lipids was higher and the stoichiometry is about 7 lipid molecules for 1 molecule of cardiotoxin which was less than the number of positive charges available in the toxin molecule (Rees and Bilwes, 1993).

**Haemorrhagin**

The term haemorrhagin was first described by Flexner and Noguchi (1902) which stated that the snake venom of *Crotalus* causes extravasations on the vessels walls. Later, haemorrhagin was described as the toxin which damages the wall of the blood vessel endothelium and capillaries (Taube and Essex, 1937; Fulton et al., 1956). Further studies stated that haemorrhagin of snake venom may be confined to cutaneous and subcutaneous tissues at the site of inoculation. It may also affect the muscle layer which leads to bleeding in the heart, brain, lung, intestine, kidneys and other organs.

Snakes belonging to the genera *Bothrops*, *Lachesis*, *Viperid*, and *Crotalus* can induce haemorrhage, whereas *Micrurus* and *Naja naja* are not. It has been extensively demonstrated that those components capable of provoking haemorrhagic disturbances are often different from those with proteolytic activity (Ohsaka, 1979; Guitirrez and Bolanos, 1980; Queiroz et al., 1985).

Some haemorrhagic principles have also been isolated from snake venom of *Bothrops*, *B. jararaca*, *B. neuwedi*, *Agkistrodon acutus*, *C. atrox*, *C. basiliscus basiliscus*, *Ophiophagus hannah* and *Vipera russellii* (Mandelbaum
et al., 1976; Tan and Saifuddin, 1989; Molina et al., 1990; Chakravarty et al., 1993). Hemorrhage is one of the most pronounced, basic effects of Crotalid snake envenomation but in recent times several hemorrhagins have been isolated from the viperid and elapid snake venom.

**Haemorrhagins from *Agkistrodon acutus***: So far nine hemorrhagin principles have been isolated from the venom of *Agkistrodon acutus* which is found in China and Taiwan (Mori et al., 1984). Five hemorrhagic toxins were isolated from this snake till 1984. All the toxins induced proteolytic activity on casein and were designated as \( A_{c1}, A_{c2}, A_{c3}, A_{c4} \) and \( A_{c5} \) proteinases. All the pure lethal hemorrhagin toxins were inhibited by EDTA, cystein and 1,10-phenanthroline.

Hemorrhagic toxin is believed to be directly related to metal zinc (Nikai et al., 1984). They determined the zinc content of \( A_{c1} \) proteinase and compared with hemorrhagic toxin from *Crotalus atrox* venom. Proteolytic and hemorrhagic activity before and after removal of zinc was compared and was found to be inactive once the zinc was removed. Thus it was concluded that all hemorrhagic toxins possesses proteolytic activity and zinc regardless of the geographical origin of the venom.

A fibrinolytic principle was isolated from the venom of *Agkistrodon acutus* having a molecular weight of 24,100 dalton with caseinolytic and fibrinolytic activity (Ouyang and Huang, 1976a and 1976b). The fibrinolytic principles were reported to have hemorrhagic activity. Three hemorrhagic toxins with proteolytic activity were purified from the same venom and designated as Aa-hemorrhagin I, II and III (Xu et al., 1981).

**Haemorrhagins from *Bothrops* species**: Different studies resulted in the isolation of five hemorrhagins from different species of *Bothrops* (*B. jararaca, B. moojeni, B. neuwiedi*). Mandelbaum et al., (1976) isolated, purified and characterized the hemorrhagic toxin from *Bothrops jararaca* venom. The toxin was designated as HF-2 having molecular weight 50,000 daltons and possessed proteolytic activity. Bothropsin was isolated and characterized by Mandelbaum et al., (1982) and was found to have proteolytic activity. A hemorrhagic
principle metalloproteinase was purified from Bothrops asper venom which possessed weak haemorrhagic activity having a molecular weight 24,000 (Gutierrez et al., 1995).

**Haemorrhagins from Crotalus atrox:** Five haemorrhagic toxins were isolated and characterized from the venom of Crotalus atrox (Bjarnason and Tu, 1978). They also showed the importance of zinc in the haemorrhagic activity. The toxins possessed proteolytic activities and were designated as Ht-a,b,c,d and e.

Two haemorrhagic toxins were isolated and characterized from Crotalus basiliscus basiliscus (Molina et al., 1990). The molecular weight of the toxins were 27,000 and 27,500 respectively and had proteolytic activity. The toxins were found to hydrolyze azure, casein, collagen and fibrin.

**Haemorrhagins from Trimeresurus species:** Two haemorrhagic toxins were isolated from this snake venom (Ohsaka et al., 1960; Takahashi and Ohsaka, 1970). Both the toxins were reported to be nonproteolytic on casein. However, protease activity of both the toxins on β-chain of insulin was demonstrated (Nikai et al., 1985). A major hemorrhagin was purified from the venom of the Thai green pit viper (Trimeresurus purpureomaculatus). The purified hemorrhagin was reported to express proteolytic activity. Both hemorrhagic and proteolytic activities were inhibited by EDTA, suggesting that the hemorrhagin is a metalloprotease (Khow et al., 2002).

**Haemorrhagins from Ophiophagus hannah:** A proteolytic haemorrhagic toxin also called 'hannah toxin' was isolated from O. hannah (Tan and Saifuddin, 1989). This toxin was isolated by gel electrophoresis, G-200 gel filtration and DEAE sephadex chromatography. The haemorrhagic and proteolytic activity were inhibited by EDTA and 1,10 phenanthroline.

**Haemorrhagins from Vipera species:** Three haemorrhagic toxins were isolated from the venom of Vipera palestinae (Ovadia, 1978). They are designated as HR-1, HR-2 and HR-3. All had a molecular weight of 60,000 daltons. HR-1 is a basic glycoprotein having minimum haemorrhagic dose of 0.2μg.
HR-2 is a weakly acidic protein of a higher haemorrhagic dose of 0.4μg. Both these toxins have gelatinase and caseinolytic activity, but HR-3 was not affected by these two substrates.

A haemorrhagic factor was isolated from *Vipera aspis aspis* having a molecular weight of 67,000 daltons. It contained 552 amino acid residues. Haemorrhagic activity was found to be lost by metal chelators and reductants. Proteolytic activity was observed on dimethyl casein (Komori and Sugihara, 1988).

Five haemorrhagins were isolated from *Vipera ammodytes ammodytes* using ion exchange and gel filtration chromatography. All the toxins showed proteolytic activity and was inhibited by EDTA (Fox et al., 1986). Two hemorrhagic proteins, VaH₁ and VaH₂, have been purified from *Vipera ammodytes ammodytes* venom. They are monomeric glycoproteins of an apparent molecular mass of 70kDa. VaH₁, which was characterised in detail, showed maximum activity at pH 7.5. Ethylenediaminetetraacetic acid eliminated the proteolytic as well as the hemorrhagic activity of VaH₁ while iodoacetamide, phenylmethylsulfonyl fluoride and pepstatin A, inhibitors of cysteine, serine and aspartic proteinases respectively, had no effect. VaH₁ is therefore a metalloproteinase whose hemorrhagic activity was very likely the result of its proteolytic activity. VaH₁ is a fibrinogenase, hydrolysing exclusively the A-chain of fibrinogen (Leonardi et al., 2002).

A haemorrhagic toxin was purified from the venom of *Vipera russellii* and was designated as VRH-1. It was found to be a protein of molecular weight 22,000 and contained one mole of Mg²⁺. The toxin showed haemorrhagic activity on lungs, conjunctiva and brain (Chakraborty et al., 1993).

**Mechanism of action of the haemorrhagins:** It has been reported that the haemorrhagic toxins did not differ much in their mechanism of action. They acted on

1. vascular permeability causing simultaneous release of erythrocytes and albumin and
2. on the blood vessel wall, damaging the junction between the endothelial cells.
In other words, the mode of action was initially attributed to an enzymatic rupture of the basal membrane followed by vascular permeability.

Haemorrhagins are metalloproteinases which require Ca\(^{2+}\) or Zn\(^{2+}\) for their activity. Many affect clotting mechanism and also hydrolyze fibrin clots or degrade fibrinogen (Nikai et al., 1984; 1985; Bjarnason et al., 1988). Haemorrhagic toxin may be myotoxic, can increase serum creatine kinase levels, disrupt capillaries and basement membrane preparation (Ownby et al., 1978; Fabiano and Tu, 1981; Mori et al., 1987; Bjarnason et al., 1988). Molecular weight of the haemorrhagins vary between 25,000 to 65,000 daltons (Mori et al., 1987; Bjarnason and Fox, 1988-89). The haemorrhagic activity was sensitive to EDTA, cysteine and metal ions like calcium and magnesium. The haemorrhagic activity was stimulated by the different enzymes found in the snake venom like protease and fibrinogen (Minta et al., 1977; Rael and Jones, 1983; Bjarnason and Fox, 1988). The complement system which enhances permeability is mainly affected (Minta et al., 1977). They suggested that the proteinase might have generated anaphylotoxins which degranulate mast cells and mediate vascular permeability. The exact mechanism of action is yet to be pinpointed.

**Inhibitors of haemorrhagins**: The naturally occurring inhibitors of haemorrhagins are \(\alpha_2\) macroglobulin and serpins (serine proteinase inhibitors). The former is a large tetrameric protein with a broad specificity towards various classes of proteinases (Starkey and Barrett, 1977). Serpin inhibits the action of serine proteinase (Travis and Salverson, 1983; Carrell and Travis, 1985). It is well known that most of the haemorrhagins are activated in the presence of metalloproteins which are inhibited by serpins (Kress and Parosky, 1978). In other studies, several inhibitors found from the snake venom of *C. atrox* and *C. adamanteus*, are proteinase II and a proteinase respectively (Kress et al., 1983; Kress, 1986). Several antihaemorrhagic factors, were also reported by Omori Satoh et al., (1972) and Ovadia (1978). They reported that the serum of *T. flavoviridis* and *Vipera palaestinae* possessed antihaemorrhagic activity. The antihaemorrhagic factor found in the serum of different animals (snake, mongoose, hedgehog, woodrats etc) has been observed (Perez et al., 1978; Menchaca and Perez, 1981). Snake venom neutralizing factors are present in...
their bloods which specifically inhibit the toxins such as haemorrhagins, proteolytic enzymes etc. Neutralizing factors have been isolated from several animal species like opossums, mongoose, woodrat and hedgehog (Werner and Vick, 1977; Bjarsnason and Fox, 1988). They possess antihaemorrhagic activity.

Several synthetic compounds like EDTA, cysteine and 1,10 phenanthroline inhibited the haemorrhagic proteinases. They mainly inhibited the functional group present in the haemorrhagin. Monovalent snake venom antiserum is another important factor possessing antihaemorrhagic activity (Lomonte et al., 1992) even though the polyvalent antiserum did not provide enough protection against venom induced haemorrhage.

Snake venom and fibrinogen

Thrombin like enzymes (TLE) : Thrombin like activity is mostly found in venoms of Crotalidae. They are absent in elapidae and hydrophidae venoms. TLE are esterases inhibited by hydroxyl group (serine) reagent (diisopropyl fluorophosphate). TLE is not affected by heparin-antithrombin III with the exception of the TLE from the venoms of Agkistrodon contortrix (Herzig et al., 1970; Guan et al., 1991). From the venom of Trimeresurus jerdonii, a distinct thrombin-like enzyme, called jerdonbin, was purified which clots fibrinogen (Lu et al., 2000). A thrombin-like enzyme, named elegaxobin, was purified from the venom of Trimeresurus elegans. It was found to clot only rabbit fibrinogen whereas bovine and human fibrinogen were not affected (Oyama and Takahashi, 2000). Calobin II, is a thrombin like enzyme from the venom of Agkistrodon caliginosus. This enzyme acted on fibrinogen to form fibrin and also exhibited arginine esterase activity. Amino acid sequencing of the N-terminal region established a primary structure composed of Val-Ile-Gly-Gly-Asp-Glu-Cys-Asn-Ile-Asn-Glu-His-Arg-Phe-Leu-Val-Ala-X-Tyr. This sequence showed a high homology with other thrombin-like enzymes, such as ancrod, batroxobin and gyroxin (Cho et al., 2001). Another thrombin-like enzyme and a fibrinolytic serine protease were purified to homogeneity from the venom of a Korean snake Agkistrodon saxatilis emelianov by Koh et al., 2001.
Snake venom and direct fibrino(geno)lysis: Snake venom enzymes also affect direct fibrino(geno)lytic activity which led to in clottable fibrinogen degradation products in contrast to the thrombin like enzymes. Snake venom components can be classified into:

i) Digestion of α-chain of fibrinogen: The venoms of *T. mucrosquamatus*, *A. acutus*, *T. gramineus*, *C. atrox* and *N. nigricollis* contains the enzyme which digested α chain of fibrinogen.

ii) Digestion of β chain of fibrinogen: The venoms of *T. mucrosquamatus*, *T. gramineus* and *C. atrox* were found to possess enzymes which digested β chain of fibrinogen.

iii) Digestion of γ chain of fibrinogen: Only one venom component was isolated from *C. atrox* which digested the γ chain of fibrinogen (haemorrhagic toxin). (Nikai et al., 1984).

Snake venom and plasminogen activator secretion: The venoms of *C. atrox* and *C. adamanteus* was reported to induce the release of plasminogen activator from human erythrocytes, platelet polymorphonuclear leucocytes, endothelial cells and smooth muscle. The stimulating agent increased both tissue plasminogen activator and urokinase intracellularly (Kirschbaum et al., 1988).

Snake venom and thrombin formation: Different snake venoms contain prothrombin activator enzymes. Venom of *Echis carinatus* has prothrombin activators which are independent of other coagulation factors, that of taipan snake is dependant on phospholipids plus CaCl₂, some are dependant on phospholipids plus CaCl₂ and factor Va. Prothrombin activators of *B. jararaca* are dependant on unknown co-factors.

Snake venoms and Factor-X activator: Factor X activator has been found in the venoms of *V. russelli*, *B. atrox* and *Crotalus viridis helleri* (Schiefman et al., 1960, Denson, 1969).

Snake venom and Factor V and Factor XI activation: *Vipera russelli* venom contains a serine proteinase (RVV-V) besides Factor X activator. Thrombocytin found in *B. atrox* venom activates Factor V besides being a platelet aggregation inducer (Nescheim et al., 1981).
**Snake venom and platelet aggregation**: Davey and Luscher (1965) reported for the first time that some snake venoms possessed platelet aggregation inducer. Platelet aggregation inducer was isolated and purified from the venom of *T. okinavensis, B. atrox,* and *C. horridus horridus* (Schmaier et al., 1980). Venom principles of *T. mucrosquamatus* and *A. rhodostoma* also contain platelet aggregation inhibitors (Ouyang, 1985a) by acting through α-fibrinogenase, digesting 5'-nucleotidase and fibrinogen receptor antagonist.

**Enzymes**

Enzymes are the major components of snake venom. They are primarily responsible for the numerous pathophysiological conditions induced due to snakebite. It is generally believed that the snake venom enzymes act in the following manner:

- **Proteinase**, hyaluronidase, phospholipase, arginine ester hydrolases, fibrinogenase affect the local loss of capillary integrity and cause tissue necrosis (Slotta, 1955; Kaiser and Michael, 1958; Suzuki and Iwanaga, 1970; Markland, 1991)

- **Proteinases**, phospholipase A and fibrinogenase disturb the haemostatic system (Meaume, 1966; Markland, 1991; Loayza et al., 1994) and induce hypotension and pain due to the release of vasoactive components by kinin releasing enzyme, kininogen (Suzuki and Iwanaga, 1970; Diniz and Oliveira, 1992).

- **5'-nucleotidase**, phosphodiesterase, cholinesterase, L-amino acid oxidase and fibrolase are the other enzymes which are proposed to be toxic.

More than thirty enzymes have been found in snake venom, twelve are found in almost all venoms, in variable concentrations. The elapidae venom is characterized by acetylcholinesterase, which is absent in viperidae and crotalidae venom (Zellar, 1950a and b). Viperidae and crotalidae venoms possess thrombin-like enzymes, fibrinolytic enzymes, kininogen and procoagulants which are absent in elapidae with the exception of King Cobra venom. The King Cobra venom contains proteolytic and arginine ester hydrolyzing enzymes (Mebs, 1970). Phospholipase A₂ is one of the major components of snake venom.
It is present in both *viperidae* and *elapidae* snakes venoms. The enzyme constituents of viperid and crotalid venoms are quite similar with no remarkable difference in the content of PLA₂, endopeptidase, phosphodiesterase, nucleotidase and hyaluronidase enzymes. Some of the enzymes found in snake venoms are mentioned below:

a) **L-amino acid oxidase**: This enzyme is detected in almost all snake venoms except white venom (*Demsonia lextiles* and *Vipera amodytes*). It contains FAD as prosthetic group which are responsible for the yellow colour of venom. One amino acid oxidase was purified from *O. hannah* which produced platelet aggregation (Zhao et al., 1994).

b) **Enzyme acting on phosphate esterases**: They are of three types which act on the hydrolysis of phosphate ester (Lee, 1979). They mainly act on nucleic acid to produce nucleosides.

1. **Endonuclease**: Presence of ribonuclease and deoxyribonuclease has been reported (Delezenne and Morel, 1919; Taborda et al., 1952a and b) in the venoms of *B. atrox*, *C.adamantens*, *C.atrox*, *V.russellii*, and *Naja oxiana*.

2. **Phosphodiesterase**: This enzyme is widely distributed in all the four groups of snakes except Bungarus family. They are also capable of hydrolyzing both DNA and RNA.

3. **3'-5' Nucleotidase**: It is a specific phosphomono-esterase found in almost all the venoms. Its activity is relatively low in elapidae compared to that of *viperidae* and *crotalidae* venoms (Mebs, 1970). This enzyme hydrolyses the phosphate mono ester which links with position 5' of DNA and RNA.

c) **Enzyme acting on glycosyl component**: The following are the enzymes which cleaves the glycosyl components.

1. **Hyaluronidase**: This enzyme catalyzes the cleavage of internal glycoside bonds of certain acid mucopolysaccharides of animal connective tissues like sodium hyaluronic acid and sodium chondroitin sulphate A and C (Lee, 1979). Concentration of this enzyme is higher.
in *viperidae* and *crotalidae* than in *elapidae*. The enzyme mainly acts as a spreading factor, because hydrolysis of hyaluronic acid facilitates toxin diffusion into the tissue of the snakebite victim. Recently, a hyaluronidase was isolated and characterized from the venom of *Agkistrodon contortrix* (Kudo and Tu, 2001).

2. **Heparinase like enzyme**: This group of enzymes act as a powerful anticoagulant by interacting with antithrombin III contained in blood plasma found mainly in crotalids (Devi and Copley, 1971). They mainly act by splitting glycosidic bonds of polysaccharides.

d) **NAD- Nucleosidase**: This enzyme catalyses the hydrolyses the nicotinamide N-ribosidic linkage of NAD. Its presence in snake venom of *Bungarus fasciatus* was first reported by Bhattacharya in 1953. The venom of all six members of *Agkistrodon* genus contained NAD nucleosidase activity.

e) **Enzyme acting on peptide bonds**: Several enzymes like proteases including endopeptidases, peptidases, proteinase, arginine ester hydrolase, prothrombin activator, Factor X activator, fibrinogenase and kininogenase which act on peptide bonds are found in snake venom. All these enzymes except peptidase are present mainly in viperidae and crotalidae venoms.

1. **Endopeptidase**: This enzyme is found mainly in *crotalidae* and *viperidae* venoms. Some *elapidae* like *Haemachatus haemachatus* and *Ophiophagus hannah* contains this enzyme in lower proportions. The enzyme endopeptidase hydrolyzes peptide bond with leucine and phenylalanine residues.

2. **Peptidase**: Peptidase is prominent in *elapidae* venom showing powerful hydrolytic activity towards oligopeptides.

3. **Arginine ester hydrolase**: This enzyme is present in *crotalidae* and *viperidae* venom but absent in *elapidae* and *hydrophidae*. Arginine ester hydrolase produces haemorrhage, necrosis and disturbs hemostatic system.

4. **Kininogen**: Prominent in *crotalidae* and *viperidae* venom, kininogen causes the fall of blood pressure and induces severe pathogenesis disturbing hemostatic system. It is capable of releasing bradykinin and cleaves some synthetic chromogenic peptides.
f) **Enzyme acting on carboxylic ester bonds**: This enzyme causes severe pathophysiological disturbances in snakebite victims. It is present in all the four groups viz. *viperidae, crotalidae, elapidae* and *hydrophidae*.

*Acetylcholinesterase* is found in *elapidae* and *hydrophidae* and absent in *viperidae* and *crotalidae* venoms. The presence of this enzyme was first reported by Iyengar *et al.*, (1938). Its concentration is very high in the cobra venom.

g) **Phospholipase**: There are three phospholipase enzymes found in snake venom. These are Phospholipase A$_2$ (PLA$_2$), Phospholipase B and Phospholipase C. PLA$_2$ enzyme hydrolyzes 3Sn-phosphoglycosides at the 2-position. It is abundant in snake venom of all the groups viz. *viperidae, crotalidae, elapidae* and *hydrophidae*. These enzymes show a wide variety of toxicity like presynaptic and postsynaptic toxicity, myotoxicity, cardiotoxicity, platelet aggregation, hemolytic, anticoagulant activity and edema formation (Kini and Evans, 1990). More than a hundred PLA$_2$ enzymes have been isolated and characterized for their pathophysiological and biochemical properties. The enzyme phospholipase C haemolyses the red blood cells which can lead to death, dermonecrosis and inflammation. (Berk *et al.*, 1987). A basic phospholipase A$_2$ (PLA$_2$) from the venom of *Agkistrodon halys pallas* was purified. It was found to be a potent hemolytic toxin and anticoagulant (Zhao *et al.*, 2000). Another basic phospholipase A$_2$ (VRV-PL-VIIIa) was isolated from *Vipera russelli* venom. It was found to induce multiple toxic effects including neurotoxicity, myotoxicity, edema and hemorrhage. This phospholipase A$_2$ has been extensively characterized for its pharmacological properties (Uma and Gowda, 2000).
Section 3
PATHOPHYSIOLOGY OF SNAKEBITE
Of the 2500-3000 species of snakes distributed world-wide, about 500 are venomous. The major families in the Indian subcontinent are: *Elapidae* which includes common Cobra, King Cobra and Krait, *Viperidae* which includes Russell’s viper, Pit viper and Saw-scaled viper and *Hydrophiidae* (the sea snakes) (Philip, 1994). Of the 52 poisonous species in India, majority of bites and consequent mortality is attributable to 5 species viz. *Ophiophagus hannah* (King cobra), *Naja Naja* (common Cobra), *Vipera russellii* (Russell’s viper), *Bungarus caeruleus* (Krait) and *Echis carinatus* (Saw-scaled viper). There are 14 venomous species in Nepal. These include pit vipers (5 species), Russell’s viper, kraits (3 species), Coral snake and 3 species of Cobra including the King Cobra.

Venom is produced and stored in paired glands below the eye. It is discharged from hollow fangs located in the upper jaw. Fangs can grow up to 20 mm in large rattlesnakes. Venom dosage per bite depends on the elapsed time since the last bite, the degree of threat the snake feels, and the size of the prey. The nostril pits respond to the heat emission of the prey, which may enable the snake to vary the amount of venom delivered. Coral snakes have shorter fangs and smaller mouths. This allows them less opportunity for envenomation than the crotalids, and their bites more closely resemble chewing rather than the strike for which the pit vipers are famous.

Venom is mostly water. Snake venom, the most complex of all poisons is a mixture of enzymatic and non-enzymatic compounds as well as other nontoxic proteins including carbohydrates and metals. There are over 20 different enzymes including phospholipases A₂, B, C, D hydrolases, phosphatases (acid as well as alkaline), proteases, esterases (acetylcholinesterase), transaminase, hyaluronidase, phosphodiesterase, nucleotidase and ATPase and nucleosidases (DNA & RNA) (Philip, 1994). The pathophysiologic basis for morbidity and mortality is the disruption of normal cellular functions by these enzymes and
toxins. Specific details are known for several enzymes as follows: (1) hyaluronidase allows rapid spread of venom through subcutaneous tissues by disrupting mucopolysaccharides; (2) phospholipase A2 plays a major role in hemolysis secondary to the esterolytic effect on red cell membranes and promotes muscle necrosis and (3) thrombogenic enzymes promote the formation of a weak fibrin clot, which in turn, activates plasmin and results in a consumptive coagulopathy and its hemorrhagic consequences.

Enzyme concentrations vary among species, thereby causing dissimilar envenomations. Copperhead bites generally are limited to local tissue destruction. Rattlesnakes can leave impressive wounds and cause systemic toxicity. Coral snakes may leave small wounds that later result in respiratory failure from the typical systemic neuromuscular blockade. There is considerable variation in the proportion of venom constituents in a single species due to geographical distribution, season of the year and also ageing of the snake.

**Epidemiology of snakebite**

Snake bite remains a public health problem in many countries 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 5 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). To complicate matters further, accurate records to determine the exact epidemiology or even mortality in snake bite cases are also generally unavailable (Philip, 1994). Hospital records fall far short of the actual number owing to dependence on traditional healers and practitioners of witchcraft etc. It has been reported that in most developing countries, upto 80% of individuals bitten by snakes first consult traditional practitioners before visiting a medical centre (Chippaux, 1988). Owing to the delay several victims die during transit to the hospital. Nevertheless, Swaroop reported about 200,000 bites and 15,000 deaths in India due to snake bite poisoning as far back as 1954 (Swaroop and Grab, 1954). Based on an epidemiological survey of 26 villages with a total population of nearly 19,000 individuals in Burdwan district of West Bengal state in India,
Hati et al, worked out an annual incidence of 0.16% and mortality rate of 0.016% per year (Hati, et al, 1992). In Sri Lanka, the overall annual mortality from a single venomous species ranges from 5.6 per 100,000 to as high as 18 per 100,000 in some areas (Sawai, 1984). Myanmar seems to have the highest mortality in Asia and 70% snakebites are by Russell’s viper (Naing, 1985). However, this may only reflect a better reporting system prevalent in that country. Maharashtra, one of the states of India with the highest incidence, reported 70 bites per 100,000 population and mortality of 2.4 per 100,000 per year. The other states with a large number of snakebite cases include West Bengal, Tamil Nadu, Uttar Pradesh and Kerala (Gaitonde et al, 1980). It has been estimated that 150 to 200 snakebite related deaths occur annually in Nepalese hospitals (Joshi et al, 1997). The WHO estimated over 20,000 cases and 1000 deaths from snakebite in Nepal (WHO, 1987). While all age groups are affected by snakebite, the large majority (90%) are males aged 11-50 years. The predominance of male victims suggests a special risk of outdoor activity. The high incidence of snake bite between 0400 hours to midnight corresponds well with the period of maximum outdoor activity observed in most studies. The incidence of snake bite shows a distinct seasonal pattern closely related to rainfall and temperature which compels the reptiles to come out of their shelter (Hansdak, 1998). Among the host factors, people involved in occupations and/or lifestyles requiring movement in dense undergrowth or undeveloped land, are the worst affected. These include farmers, herders and hunters (Warrel, 1987) and workers on development sites. Paul reported an incidence of 7-15 percent in children less than 10 years. Bites are maximal in lower limbs (about two thirds) (Warrel, 1996) with 40 percent occurring in feet alone.

Morbidity and mortality resulting from snakebite envenomation also depends on the species of snake involved, since the estimated ‘fatal dose’ of venom varies with species. In the Indian setting, almost two-thirds of bites are attributed to Saw-scaled viper (as high as 95% in some areas like Jammu) (Bhat, 1984), about one fourth to Russell’s viper and smaller proportions to cobra and kraits (Saini, 1984). In Sri Lanka, Vipera russellii accounts for 40% of bites and Naja naja for another 35% (Sawai, 1984). Vipera russellii alone accounts for 70% bites in Myanmar (Naing, 1985). Among the various species,
the average yield per bite in terms of dry weight of lyophilized venom is 60 mg for cobra, 63 mg for Russell’s viper, 20 mg for krait and 13 mg for saw scaled viper. The respective ‘fatal doses’ are much smaller viz 12 mg, 15 mg, 6 mg and 8 mg (Reddy, 1979). However, clinical features and outcomes are not as simple to predict because every bite does not result in complete envenomation (Reid, 1982). Epidemics of snake bite following floods owing to human and snake populations getting concentrated together have been noted in Pakistan, India and Bangladesh.

In Burma, snakebite has been the country’s fifth most important cause of death, attributed mainly to Russell’s viper. In Thailand, the reported number of cases of snakebite per 1,00,000 population per year increased from less than 4 (1979-81) to 10 in 1988. It seemed likely that the increase was because of better reporting rather than more snakebites in the country. Though no data are available in Vietnam, bites by *C. rhodostoma* are common in rubber and coffee plantations in South West Vietnam. Cobra bites also cause a number of deaths.

In China, the Hainan and Taiwan islands are worst affected by incidences of snakebite. In Guangxi Zhuang autonomous region, 974 cases of snakebites were recorded with 23 deaths, in 1990 (Sawai *et al.*, 1992). The cause of bites were mainly *N. atra* (27.4%), *T. stejnageri* (20.9%) and *T. murosquamatus* (13.4%). In Japan, 5,488 cases with 50 deaths from Habu bites (*T. flavoviridis*) between 1962-70 were recorded (Toriba and Sawai, 1990). The mamushi (*A.b.blomhoffii*) commonly found in the larger islands, bites more than 500 people every year with a mortality rate of 0.9%. In Malaysia, *C. calloselasma*, cobras and *T. purpureomaculatus* are responsible for most of the venomous bites while cobras are mainly responsible for deaths in Philippines. Statistical data of 1968 show 294 deaths where 327 were in Luzon and 36 in Mindanao area (Sawai *et al.*, 1975).

**Clinical features of snake envenomation**

The clinical features of snakebite are distinct, with a wide range of severity. The amount of venom injected will vary depending on the age and health of
the snake. Not all snakebites are fatal and many do not manifest any symptoms at all. In a study of 432 snakebites in North India, Banerjee noted that 80% of the victims showed no signs of envenomation (Philip, 1994). This is also corroborated in an earlier report by Saini, where only 117 cases out of 200 developed symptoms (Saini et al., 1984). It may be due to either a very high incidence of dry bite (Strong et al., 1997; Silveria, 1995) or the amount of venom injected is insufficient (Hagwood, 1998). Dry bite will manifest only local pain and irritation, whereas the most severe envenomations may display profound coagulopathy, hypotension and shock, or even death. Pain is a feature of all bites, begins almost immediately and is a persistent feature.

The most common and earliest symptom following snake bite (poisonous or non poisonous) is fright (Reid, 1979). Owing to fright, a victim attempts ‘flight’ which unfortunately results in enhanced systemic absorption of venom. These emotional manifestations develop extremely rapidly (almost instantaneous) and may produce psychological shock and even death. Fear may also cause transient pallor, sweating and vomiting. Many patients experience various nonspecific symptoms. Nausea and vomiting are common, and diarrhea may be a feature as well. Weakness, diaphoresis, malaise, perioral or digital paraesthesias may occur. The time of onset of poisoning is similar in different species. Cobra produces symptoms as early as 5 minutes (Paul, 1993) or as late as 10 hours (Reid, 1979), after the bite. Vipers take slightly longer - the mean duration of onset being 20 minutes (Paul, 1993). With the possible exception of the psychological trauma of being bitten, local changes are the earliest manifestations of snake bites. Features are noted within 6-8 minutes but may have onset up to 30 minutes (Reddy, 1980; Reid, 1983). There is local pain with radiation and tenderness followed by edema. Envenomation leads to increase in the capillary permeability which may cause loss of blood and plasma volume into the extravascular space. This accumulation of fluid in the interstitial space is responsible for edema. Tingling and numbness over the tongue, mouth and scalp and paraesthesias around the wound occur mostly in viper bites (Reddy, 1980). Regional lymphadenopathy has also been reported (Trevett et al., 1994). Secondary infection including tetanus and gas gangrene may also result (Sathyananathan, 1993).
Based on the predominant constituents of venom of a particular species, snakes were loosely classified as neurotoxic (notably cobras and kraits), hemorrhagic (vipers) and myotoxic (sea snakes). Neurotoxic features are a result of selective d-tubocurarine like neuro-muscular blockade which results in flaccid paralysis of muscles (Paul, 1993) though cobra venom is 15-40 times more potent than tubocurarine. Ptosis is the earliest (Phillip, 1994) neuroparalytic manifestation followed closely by ophthalmoplegia. Paralysis then progresses to involve muscles of palate, jaw, tongue, larynx, neck and muscles of deglutition— but not strictly in that order (Paul, 1993). Generally muscles innervated by cranial nerves are affected earlier. Muscles of chest are involved relatively late with diaphragm being the most resistant. This accounts for the respiratory paralysis, which is often terminal. Reflex activity is generally not affected and deep tendon jerks are preserved till late stages (Phillip, 1994). Onset of coma is variable, however several cases of cobra bite progress to coma within 2 hours of bite. Symptoms that portend paralysis include repeated vomiting, blurred vision, paraesthesiae around the mouth, hyperacusis, headache, dizziness, vertigo and signs of autonomic hyperactivity (Kulkarni et al., 1994).

Cardiotoxic features include tachycardia, hypotension and ECG changes. Cardiotoxicity occurs in about 25% viperine bites and includes rate, rhythm and blood pressure fluctuations (Nayak et al., 1990). Acute myocardial infarction and tetanic contraction of heart following a large dose of cobra venom has been documented in vivo and in vitro (Hagwood, 1996).

The venom of Vipera russelli which has procoagulant activating factors V and X. Certain other venoms cause defibrinogenation by activating endogenous fibrinolytic system (Budzynski, et al., 1984; Kitchens, et. al., 1983). Besides direct effects on the coagulation cascade, venoms also can cause qualitative and quantitative defects in platelet function (Mirtschin, 1991). Bleeding may occur from multiple sites including gums (Warrell, 1996), GIT (haematemesis and melaena), urinary tract, injection sites and even as multiple

(49)
petechiae and purpurae (Reid, 1979). Subarachnoid haemorrhages were documented in 5 of 200 cases in Saini's series of patients in Jammu region (Saini, 1984). In addition cerebral haemorrhage (Mirtschin, 1991) have also been reported. Almost every species of snake can cause renal failure. It is fairly common following Russell's viper bite and is a major cause of death (Myint-Lwin et al., 1985). Renal damage may occur, usually in the form of acute tubular necrosis (ATN). ATN is usually due either to hypoperfusion secondary to hypovolemia, or rhabdomyolysis. However, cortical necrosis has been described in severe envenomations. Rarer systemic manifestations including hypopituitarism (Uberoi, 1991; Majumdar, 1992) bilateral thalamic haematoma and hysterical paralysis (Adogu, 1992) have also been reported. The effect of sea snake bite are primarily myotoxic effecting the skeletal muscle. The venom of E. schistosa myotoxin and other presynaptic toxins inhibit the choline transport system. It blocks the resynthesis of acetylcholine from choline (Tu, 1977).

While there are many factors influencing the outcome in victims of snake-bite, there is an overall agreement in the case fatality rate - generally varying from 2-10% (Paul, 1993). The mortality rate is higher in children owing to larger amount of toxin per kg body weight absorbed (Russell, 1980). There is significantly higher mortality among victims who develop neurotoxicity (Hansdak, 1998). On an average - cobras and sea snakes result in about 10% mortality (Reid, 1979) ranging from 5-15 hours following bite. Vipers have a more variable mortality rate of 1-15% and generally more delayed (up to 48 hours) (Paul, 1993).

Children overall fare worse than adults owing to greater amount of toxin injected per unit body mass. Patients bitten on the trunk, face and directly into bloodstream have a worse prognosis (Laloo et al., 1995). Victims of snake envenomation, who develop secondary infection at the site of bite fare worse than those uninfected (Russell, 1980).
CASE STUDIES

Laothong and Sitprija (2001) has mentioned 3 case studies of Malayan krait (Bungarus candidus) envenomation. All the three patients developed ptosis and generalized muscle weakness which later progressed to respiratory paralysis. All the patients showed evidence of decreased parasympathetic activity manifested by mydriasis, hypertension and tachycardia. Due to non availability of specific antivenom, the patients received assisted ventilation and supportive treatment. Two patients recovered while the third one had permanent brain damage due to anoxia from two episodes of cardiac arrest. In another report, neurological deficits like cerebral infarction was observed following viper bite. A 21 years old male developed motor aphasia and right hemiplagia within 2 hours of being bitten by viper (Panicker and Madhusudanan, 2000).

**Outcome** of snake bite cases depends upon many factors including

1. Species of the snake with corresponding species specific toxity
2. Nature of the Venom Toxin (Neurotoxicity / Haemotox/Nephrotoxicity)
3. Amount of the venom injected
4. Relative lethal potential LD₅₀ of the toxins
5. Extent of dispersion of the venom in the circulation (ligature/ wound care etc)
6. Primary wound care
7. Rapid identification of the type of toxins and quantification
8. Institution of therapy with specific antidotes
9. Close observation and supportive treatment/ intervention
10. Pre-existing physiological status of the patient

**Monitoring**: It is essential to identify the type (Elapid / Viper) species of the snake involved in order to guess the possible nature of the toxin (haemolytic / neurotoxin / nephro / cardiotoxic) the clinician need to tackle. Various commercially available electrophoresis kits are available for identification of specific snake venoms. Amount of the toxin in the circulation that is to be neutralized by anti snake venom can be precisely measured using commercially available diagnostic kits based on the principle of ELISA. Very few such kits are calibrated for Indian species of snakes and their corresponding snake venom types. High cost and infrastructural requirement limits their wide spread use in day to day clinical practice. Lack of identification of the type of snake involved by the
patient/party, limits availability of sophisticated laboratory facilities like electrophoresis and ELISA to characterize and quantify the toxin in the clinical setting. Nephrotoxicity of the patient is usually monitored by serial routine chemical and microscopic examination of the patient urine for blood/RBC/cast and protein. Serial hematocrit and total counts are employed to assess the extent of haemolysis. Bedside electrocardiogram/cardiac monitors document the arrhythmia/cardio toxicity. Bedside clinical neurological examination is used to assess focal neuro deficit. For day to day clinical practice Glasgow Coma score (GCS) is used which includes best motor, best verbal and eye opening response following verbal commands. Pupil, cranial nerves, sensorimotor, coordination and plantar and other reflexes are serially screened.

Treatment: Lyophilized ‘Anti Snake Venom’ (ASV) reconstituted by dissolving in normal saline are given via short intravenous injections in initial bolus dose followed by slow intravenous infusion in maintenance dose are continued using infusion pump or intravenous drip sets till the patient improves clinically and the markers reach their desired normal levels. Supportive care includes intravenous fluid and inotropic support to combat circulatory collapse, antibiotics to prevent infection/septicaemia from the bite wound, diuretics to increase/maintain renal clearance. Neurotoxins are often antagonized by time to time repeated injections of cholinomimetic agents like neostigmine combined with atropine(to prevent the unwanted muscarinic actions). Hemolytic episodes are tackled with blood transfusions as and when required. These toxins being immunologically active may induce acute hypersensitivity reactions along with ‘Systemic Inflammatory Response’ and may require therapy with steroids and anti allergic agents.

Case I: Ms P. Malaker, 24yrs/female from Garbeta, Midnapur, West Bengal India, was admitted in Medical College Hospital on 29th Sep 1998 following snake bite on the afternoon of 28th Sep 1998. The type of the snake was presumed to be ‘Kalchiti’ as per description reported by the family members and was suspected for possible neurotoxicity characteristic of the species.

On admission she presented with altered consciousness, responding by minimal eye opening only to pain stimulus with bilateral flaccid paralysis of all four limbs with decreased muscle tone, motor power of 2/5, and impaired
deep tendon reflexes. She had bilateral ptosis with bilaterally equal sized pupil, sluggishly reacting to light.

Clinically she had marked pallor (Hemoglobin 7.3gm/dL), tachycardia with pulse rate of 110/min, low volume rapid thready pulse with a BP of 110/60mm of Hg and a respiration of 32/minute.

She was in shock with central venous pressure of 8 with a blood gas picture showing oxygen saturation of 89% only with metabolic acidosis. She received volume support through intravenous drip in order to restore the cvp with two units of packed cell transfusion to correct her pallor and to improve her $O_2$ saturation. She received 5 ampoules of neostigmine along with 2 ampoules of atropine, 1/2 hourly - four such shots followed by four such shots at 1 hourly intervals. She was put on antibiotics (ampicillin, gentamycin, metronidazole) and moist oxygen inhalation at $\text{F}O_2$ of 4L/min.

15 x 10ml of ASV was given with minimal clinical improvement and she required intermittent positive pressure ventilatory support due to paralysis and fatigue of respiratory muscles. She expired on 3rd Oct 1998 (Fig. 15,16 and Table 1).

Case II : Mr. L. Mandal, 54yrs/male from Domjur Howrah, was admitted on 18th Sep 1998 following snake bite two days back. Type of the snake could not be ascertained.

He had increased lethargy, intense retching with progressively decreasing urine output for two days following snake bite. He was passing 230 to 250 ml of high colour urine per day for next two days. Routine urine examination revealed microscopic hematuria and albuminuria (RBC: 8-10/high power field, urine albumin 3+ , specific gravity of 1.088 along with hyaline cast). He had moderate pallor and oedema with a pulse rate of 94/minute, blood pressure of 130/82 mm of Hg and no focal neurological localizing sign/deficit. Most of his laboratory parameters were stable except the serum urea and creatinine levels were 102 and 6.1 mg/dL respectively.

Along with local wound dressing he received 10 x 10ml of ASV and his hematuria was controlled after three days. Despite adequate hydration and intravenous furosemide (Lasix) challenge he required CAPD (Chronic ambulatory peritoneal dialysis) in the nephrology unit. He was discharged after eleven days (Fig. 17,18 and Table 2).
Fig 15  Case Study I — Ms P. Malakar

Fig 16  Case Study I — Ms P. Malakar, showing symptoms of snakebite
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Fig 17  Case Study II — L. Mondal

Fig 18  Case Study II — L. Mondal, showing symptoms of snakebite
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Section 4

TREATMENT OF SNAKE BITE
The famous Hearst medical Papyrus dated around 1600 B.C. provides the oldest information on snake poisoning and its treatment. This prescription of Egyptian medicine is preserved at the University of California, Berckley. Later this knowledge of treatment was adapted by the Greeks. A more systemic study of plant, animal and mineral remedies against snakebite was described by Dioscorides in his famous book ‘De Materia Medica’ during the Roman rule. In the great Matthioli edition of Dioscorides, a considerable amount of information is available on venoms and bites of different kinds.

The first twenty five sections of the ‘seven books of Paulus Aegineta’ (Book V) (Syndenham society, London,1846) deals with venomous animals and their treatment. Records dated as early as 15th Century mentions the application of snake venom antidote or ‘Tiryaque’ by Islamic scholars prepared from plant sources. In India, folk medicines are very popular. In fact most snake bite victims in rural India depend on herbal remedies.

Henry Sewall undertook the study of immunization for the first time in 1887. He suggested that it is possible to induce venom resistance in a pigeon by injecting venom in increasing dose. Calmette finally in 1894 defined immunity and prepared the snake venom antiserum (Calmette, 1894). Till date, antiserum is the only specific treatment available for snakebite.

**Non specific treatment of snakebite**

Even today, most of the snakebite victims take resort to the non specific mode of treatment specially in rural India. There are various folk medicines which are widely used by snake charmers, medicine men or ojhas and villagers.

**Mantra (chanting of healing words)** is performed by religious men in and around the rural belts of Asia and Africa. They chant mantras and apply a
mixture of plants which is generally very unscientific. Since most of the bites are due to non venomous snakes and are not fatal, mantras and traditional healers earn good faith and reputation. Blind faith for them leads to death of the victim. Snake stones are used by ojhas in Kerala, Sri Lanka and West Bengal. It is a brownish plant seed which is believed to “draw out the venom” which is again unscientific. Certain Patent medicine are also widely used to treat snakebite victims by ancient medical practitioners. They were ‘Roxin’ a gold chloride based inhalant and ‘Tiryaque’ or antidote.

Specific treatment of snakebite

Snakebite, a major problem leads to a high incidences of death. The specific protocol of snakebite management can be discussed under the following:

a) First aid
b) Specific therapy
c) Supportive therapy

First aid or Pre hospital

Most physicians are in disagreement with regard to nature, duration and even necessity of first aid. Russell advises minimal wastage of time with first-aid measures which often end up doing more harm than good (Russell, 1980). Nevertheless, it is felt that reassurance and immobilization of the affected limb with prompt transfer to a medical facility are the cornerstones of first-aid care (Paul, 1993). Movement promotes propagation of the venom and increases risk of systemic effects prior to reaching the controlled hospital environment. Most experts also advocate the application of a wide tourniquet or crepe bandage over the limb to retard the absorption and spread of venom (Paul, 1993; Reid, 1979). The tourniquet should be tight enough to occlude the lymphatics, but not venous drainage though some also prefer to occlude the veins. It was formerly believed and therefore advocated that incision over the bite drains out venom. However, it has now been established from animal experiments that systemic venom absorption starts almost instantly; this form of ‘therapy’ is therefore being questioned (Russell, 1980; Reid, 1979). In addition to local skin, nerve or tendon damage, necrosis is promoted and increased wound infection rates may result.
Reid (1979) has advised that the wound site be minimally handled. Most authors recommend saline cleaning and sterile dressing. Some however advise that the wound be left open (Reid, 1983). There is disagreement over the use of drugs as part of first-aid care. It has been suggested that NSAIDS particularly aspirin may be beneficial to relieve local pain. Russell however dissuades use of analgesic and in particular aspirin for fear of precipitating bleeding (Russell, 1980).

Specific therapy - Snake venom antiserum

Antivenoms are prepared by immunizing horses with venom from poisonous snakes and extracting the serum and purifying it. Antivenoms or snake venom antiserum may be species specific (monovalent) or effective against several species (polyvalent). Monovalent antivenom is ideal, but the cost and non-availability, besides the difficulty of accurately identifying the offending species - makes its use less common (Warrel, 1996).

Snake venom antiserum is administered intravenously. Indications for the use of snake venom antiserum and the number of vials needed depend on the clinical state and the species of snake involved. There are specific indications for use of antivenom (Gaitonde and Bhattacharya, 1980; Warrel, 1996). Administration of only one or two vials is inappropriate. At least five vials are needed as initial therapy. It should be begun as a slow i.v. infusion of a dilute concentration with prudent increases in the drip over the following 30 minutes to the desired rate. Children generally require more antiserum per body weight than adults. There are specific indications for the use of antiserum. Every bite, even if by poisonous species does not merit its use. This caution against the empirical use of antiserum is due to the risk of hypersensitivity reactions (Theakston and Reid, 1983) which is its major drawback. Therefore, snake venom antiserum is indicated only if serious manifestations of envenomation are evident viz coma, neurotoxicity, hypotension, shock, bleeding, acute renal failure, rhabdomyolysis and ECG changes (Paul, 1993). In a study of elapid envenomation from India, victims with neuromuscular paralysis were administered anticholinesterase/ neostigmine. Four of the patients did not receive any antiserum; all survived. Of 8 who received antiserum 3 were given less than (56)
50 units, all 3 survived. The other 5 were administered more than 50 units, however 2 died. It was concluded that antiserum has no definite role in elapid envenomation (Bomb et al., 1996).

Despite widespread use of antiserum, there are virtually no clinical trials to determine the ideal dose (Thomas and Jacob, 1985). Conventionally 50ml (5 vials) is infused for mild manifestations. Moderate envenomation defined by presence of coagulation defects or bradycardia or mild systemic manifestations, merits the use of 100ml (10 vials). 150ml (15 vials) is infused in severe cases, which includes rapid progression of systemic features, encephalopathy and paralysis (Paul, 1993).

The freeze dried powder is reconstituted with 10ml of injection water or saline or dextrose. A test dose is administered on one forearm with 0.02ml of 1:10 solution intradermally. Similar volume of saline in the other forearm serves as control. Appearance of erythema or wheal greater than 10mm within 30 min is taken as a positive test. In this event, desensitization is advised starting with 0.01ml of 1:100 solution and increasing concentration gradually at intervals of 15 minutes till 1.0ml (s.c.) can be given by 2 hours. Infusion is started at 20ml/kg per hour initially and slowed down later (Paul, 1993). Antiserum is administered by the intravenous route (Paul, 1993) and never into fingers or toes (Russell, 1980).

Hypersensitivity reactions including the full range of anaphylactic reactions may occur in 3-4% of cases, usually within 10 to 180 minutes after starting infusion (Chatterjee, 1965). These usually respond to conventional management including adrenaline, antihistamines and corticosteroids. Several antivenom preparations are available internationally. In India, polyvalent antivenom prepared by C.R.I. Kasauli is effective against the 4 commonest species (Paul, 1993). Antivenom produced at the Haffkine Corporation, Parel includes more species as well. This is about 10 times as expensive as the former. The WHO has designated the Liverpool School of Tropical Medicine as the international collaborating centre for antiserum production and/or testing (Blackman and Dillon, 1992).
Supportive Therapy

Supportive therapy is vital in snakebite management. In cases of bleeding, replacement with fresh whole blood is ideal. Volume expanders including plasma and blood are recommended in shock (Paul, 1993). Early mechanical ventilation is advocated in respiratory failure. Cases of acute renal failure generally respond to conservative management. Occasional peritoneal dialysis may be necessary. Routine antibiotic therapy is not a must though use of broad spectrum antibiotics are recommended (Paul, 1993). Chloramphenicol has been claimed to be useful as a post bite antibiotic even when used orally since it is active against most of the aerobic and anaerobic bacteria present in the mouths of snakes. A study of the organisms isolated from the mouth of the Malayan pit vipers suggests that crystalline penicillin with gentamicin would also be appropriate antibiotic cover following snakebite (Kuzbari et al., 1994). Recent studies have reported the beneficial effects of intravenous immunoglobulin (i.v. Ig) in snakebite management. There are suggestions that its administration may improve coagulopathy, though its effect on neurotoxicity is questionable. A pilot study indicates that i.v. Ig with antiserum eliminates the need to repeat antiserum for envenomations associated with coagulopathy (Sellahewa et al., 1994).

Current Wyeth polyvalent crotalid antiserum is made from horse serum. Horses are injected with the venom of four different North American crotalids. The horse serum is then refined and freeze dried for use. The entire immunoglobulin molecule is used. The two Fab fragments are responsible for the binding and neutralization of venom toxins. The one Fc fragment, as well as nonspecific horse serum proteins, are thought to be responsible for immune mediated reactions (i.e. anaphylaxis) (Walter, 1999).

New crotalid antiserum from an ovine (sheep) source contains only Fab fragments. Theoretically, this type of antiserum could be given more readily and more frequently, with much lower incidence of serious reactions. This would reduce morbidity and mortality in significantly envenomated patients, and possibly provide safe treatment of symptoms in less severely envenomated patients.

There is a new antiserum called CroFab. The initial studies show very promising results. One drawback to the use of CroFab has been the recurrence
of symptoms after initial improvement. This may be related to delayed venom absorption from the bite site coupled with the relatively short half-life of the Fab fragment, unbinding of the Fab from the toxins, or from production of human anti-sheep antibodies. As yet, the product has not been studied in or approved for use in copperhead bites (Seiler et. al., 1994). A compound extracted from the Indian medicinal plant *Hemidesmus indicus* RBr (2-hydroxy-4 methoxy benzoic acid (Alam, et al., 1998) has been noted to have potent antiinflammatory, antipyretic and antioxidant properties, particularly against Russell’s viper venom (Alam et al., 1998). These experiments suggest that chemical antagonists from herbs hold promise in the management of snake envenomation, particularly when used in the presence of antiserum. Four cases of tetanus have been documented following snakebite (Russell, 1980) hence tetanus toxoid is a must.

Snakes do not generally attack human beings unprovoked. They are reputed to be more afraid of man than vice-versa. Nevertheless once bitten, a wide spectrum of clinical manifestations may result. The emphasis for treatment should be placed on early and adequate medical management. Over emphasis on first-aid can be dangerous because its value is debatable and too much valuable time is wasted in its administration.
Section 5

HERBAL TREATMENT
OF SNAKEBITE
Importance of Herbal remedies

The World Health Organization (WHO) has defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. In other words, traditional medicine is the synthesis of therapeutic experience of generations of practising physicians of indigenous system of medicine.

Fostering health by old means and new, the World Bank is supporting various projects specially in South Asia to make the best of a precious natural resource: plants used in traditional medicines and modern pharmaceuticals. As a result of these efforts, different communities are beginning to conserve and sustainably harvest plants that have provided remedies for hundreds of years and that yield ingredients for some of the most advanced medications available today. Increasing number of people are relying on herbal remedies as a principal means of preventing and curing illnesses, and several traditional medical systems are based on the use of plants. Herbal medicine is still the mainstay of about 75-80% of the world population mainly in the developing country for primary health care (Kamboj, 2000). According to the World Health Organization (WHO) the use of herbal remedies throughout the world exceeds that of the conventional drugs by two or three times (Evans, 1994). Many conventional drugs originated from plant source: a century ago, most of the few effective drugs were plant based. Example include aspirin from willow bark, quinine from cinchona bark and morphine from the opium poppy (Vickers and Zollman, 1999). There are several advantages to such systems: the plants involved are readily available, are easy to transport, and do not spoil quickly. Remedies based on these plants often have minimal side effects, and the relatively high cost of synthetic medicines in developing countries often makes traditional herbal medicines an affordable choice for the poor in these lands.
India is a storehouse of thousands of medicinal plants. Its traditional medical systems are part of a time honoured and time tested culture that still intrigues people today. A culture that has successfully used nature to treat primary and complex ailments for over 3,000 years obviously has a contemporary relevance. India’s rich tradition as evident from Ayurveda, could not have flourished for two thousand years without any scientific basis. Charak Samhita and Sushruta Samhita are the two famous treatises of Ayurveda (Lele, 1999). In an age when toxic drugs are increasingly unwelcome and when thinking people are using viable alternatives, India’s medical heritage are gradually being scientifically documented. For instance, the small coniferous Himalayan yew (Taxus baccata) has recently become a heavily traded species. It is avidly sought because it contains taxol, used to treat ovarian cancer. Emblica officinalis L., Curcuma longa L., Mangifera indica L., Momordica charantia L., Santalam album L., Swertia chirata Buch-Ham, Winthania somnifera (L.) have well defined antioxidant properties and justify their use in the past as well as the present (Scartezzi and Speroni, 2000).

Approximately 7,000 medical compounds prescribed by Western doctors are derived from plants. Forest products are found to possess therapeutic value and serve two purposes. First, the extracts can be used directly as drugs. For maladies ranging from nagging headaches to lethal contagions such as malaria, medicinal plants have provided modern society with a variety of cures and pain relievers. Secondly, their chemical structures sometimes serve as templates from which scientists and researchers can chemically synthesize drug compounds. For example, the blueprint for aspirin is derived from extracts of willow tree. Neostigmine, a chemical derived from the Calabar bean and used to treat glaucoma in West Africa, also provides the blueprint for synthetic insecticides. However, the chemical structures of most natural drugs are very complex, and simple extraction is usually less expensive than synthesis. Ninety percent of the prescription drugs that are based on higher plants include direct extractions from plants.

The World Health Organization estimates that 80 percent of the people in developing countries still rely on traditional medicine for their primary health
care needs. Without money, access to, or faith in modern facilities, indigenous people depend on shamans, herbal healers, and rainforest plants for their survival. Shamans or the medicine men also play a crucial role in helping scientists to discover the potentials of plants. As one scientist has said, 'Each time a medicine man dies, it is as if a library has been burned down.'

Out of the 250,000 identified higher plants in the world, about 35,000-70,000 have at one time or other used by some people or cultures for medicinal purpose (Ernst, 2000). Ayurveda, the Indian scripture contains over 8,000 herbal remedies. In 1st century AD the Chinese had described 365 herbal healing in Materia Medica. Today the list is over 5,800 species. Assyrians and Sumerians recorded 250 and 1,000 medicinal plants respectively. The later civilizations of Egypt, Greece, Rome, Arabia, Europe and America, all listed their plants that are used as medicine.

In the industrialised countries people are seeking alternative herbal medicine because of the side effect from the strong modern drugs. According to World Health Organisation (WHO) 70 to 90 per cent of world population especially from developing countries, use plant remedies for their health care. Many chemical substances of known structure are extracted from plants that are used as drugs all over the world. Several more medicines derived from plants are being introduced for treatment of human diseases. A recent survey, estimated that 39% of all 520 new approved drugs in 1983-1994 were natural products or derived from them. Antibacterial and anticancer drugs have also been found to be derived from natural products (Harvey, 1999).

Injury and death due to snakebite, is a major sociomedical problem in a tropical country like India. For the past hundred years, snake venom antiserum is the only treatment that is available to the victims. However, serum does not provide enough protection against venom induced haemorrhage, necrosis, nephrotoxicity and often develops hypersensitivity reactions. Serum production is also time consuming and is expensive. It needs ideal storage conditions which are not met with, specially in the rural areas. In order to overcome these drawbacks, search is on to find alternate treatment to neutralize snake venom. Since time immemorial, herbs have been used in India as medicine. Traditional
literature recommended many medicinal herbs which were widely used to treat snakebite victims (Chopra et al., 1956). So far none of these plants have been scientifically proved to be effective against snake venom induced actions.

Scientists all over the world are eager to develop a suitable antagonist from the plant source, inspite of the existence of antiserum. Research on plants as antsnae venom agents have several objectives. They are:

♦ identifying the plants which possess potent antisnake venom activity
♦ isolation and purification of the active constituents responsible for antagonizing snake venom, which might be developed as a drug
♦ the identification of the agents with novel structures and the mechanism of action which can be used in the study of snake venom antagonism.

India has a rich herbal heritage. It is a large storehouse of innumerable medicinal plants. They have been used popularly to treat various diseases. In the rural parts of India, the people are mainly dependant on the traditional healer which possesses rich cultural heritage (Kirtikar and Basu, 1935; Chopra et al., 1956). Many plants are recommended in traditional medicine as active against snakebite (Mhaskar and Caius, 1931; Chopra and Chopra, 1955; Biswas and Ghosh, 1977). So far, none of these plants have been clinically shown to be effective against snakebite.

The first scientific document of using a plant against snakebite was published in 1921 by Knowles. They noted several plants which were used as snakebite antidotes by the medicine men. Lack of proper knowledge about lethal and sublethal dose of the snake venom and its dependence on the body weight of the victim, provided with misleading results. Mhaskar and Caius (1931) had similar failures when they found that none of the 314 plants that were mentioned in traditional literature of India, Africa, Brazil and the Americas were effective as snake venom antidote.

Biswas and Ghosh (1977) mentioned that fifty two species of higher plants from sixteen families are active against snake venom. Andrographis paniculata (Acantheceae) locally called kalmegh was reported to be used as a snakebite antidote (Nazimudeen et al., 1978). The water soluble portion of its ethanolic
root extract showed a life prolonging effect on mice after Cobra (Naja naja) venom exposure. The cause of death was due to respiratory failure and neuromuscular blockade, which was antagonized by the extract.

Aerial parts of Diodia scandens (Rubiaceae) was used as snake venom antidote (Mittal et al., 1981) and reported its capacity as antihistaminic and antiserotonic activity. Gymnema sylvestre (Asclepiadaceae) root is mentioned in literature as a snake venom antidote. Kini and Gowda (1982) investigated the inhibitory effect of potassium gymnate of Cobra (Naja naja) venom ATPase. Aqueous extract of the root of Tanacetum umbelliferum Boiss (Bozidan) was effective against viper (Vipera russellii) venom induced lethal effect (Tajuddin et al., 1989). The plants neutralized venom induced defibrinogenating action. Plants of Aristolochiaceae family was reported to be effective against snake bite. Root of Aristolochia tagala was found to be an effective antidote of Naja naja venom (Nair et al., 1994). Aristolochia indica, Gymnema sylvestre, Moringa oleifera were reported to have antisnake venom activity and were widely used by the Yannadi tribes of Chittoor district of India (Sudarsanam and Sivaprasad, 1995). Hemidesmus indicus root extract has been reported to be potent against snake venom induced activity (Alam et al., 1996). It was found to effectively neutralize viper venom induced lethal, haemorrhage, coagulant and anticoagulant activities. Various plant species are used to treat snakebite victims in different countries. Roy Chaudhury (1999) has listed few plants with antisnake venom activity. In India the plants popularly used are the leaves of Andrographis paniculata, roots of Gloria superba, leaves of Tephrosia purpurea, roots or leaves of Achryanthus indica, seeds and roots of Crotolaria laburnifolia. In Myanmar, some other plant species are used like Harrisonia perforate, Leela aspera, and Ruella tuberosa. The red flowers of Clinacanthus noretanus and yellow flowers of Barleria lupulina are used to treat snakebite in Thailand (Roy Chaudhury, 1999).

Scientists all over the world have exploited our natural resources to search for a snake venom antagonist. Plants mentioned in traditional medicines have been studied by Leung, 1980; Morton, 1981; Havsteen, 1983; Duke, 1985; Mors, 1991 and Martz, 1992. Numerous flowering plants were found to possess...
antisnake venom activity by Houghton and Osibogun (1993). Various plants selected from folk medicine were also recommended as snakebite antidotes (Pariera et al., 1994). Attempts were made to investigate the scientific validity of these information against venom inhibition.

Okonogi et al., (1979) studied the detoxifying effect of Persimmon tannin extracted from *Diospyros kaki* Thumb (Ebenaceae) against *Agkistrodon halyis blomhoffi* snake venom in Japan. Persimmon tannin was extracted from the unripe fruit and tested against the snake venom activity. The antagonistic effect of the same tannin was also found against *Laticauda semifasciata* Reinwardt (Erabu sea snake) and against haemorrhagic effect of the venom of *Trimeresurus flavoviridis* Hallowell (Habu land snake).

Curcuma species (Zingiberaceae) was reported to neutralize the proteolysis independent Cobra neurotoxin (Cherdchu, 1977; 1983). The rhizome of the plant called ‘Wan Ngu’ is orally administered to the victim of Cobra bite and was found to be effective.

Numerous Chinese herbs are reported to possess antisnake venom activity (Ma et. al., 1982). The Giant Taro has been reported to neutralize *Agkistrodon acutus* venom (Wu, 1981). One of the important Chinese herbal drugs called ‘Ma-tou-Ling’ (*Aristolochia shimadai* Hay radix) is used for emetic and expectorant purposes as well as against snakebite. The crude extract of *Aristolochia radix* appeared to be effective against crotalid venom but not elapids (Tsai et al., 1980). It was reported that allantoin and aristolochic acid isolated from *Aristolochia shimadai* neutralized both *Naja naja* and *Bungarus multicinctus* induced haemorrhagic activity. *Picrasma quassioides* was also reported to have anti snakebite action (Liang, 1985).

Prenylated pterocarpans, cabenegrin AI and AII was isolated from the ethanolic root extract of Cabeca de negra (Nakagawa et al., 1992). These compounds neutralized *Bothrops atrox* venom induced cardiorespiratory and lethal action. Methanolic extracts of the stem of *Schumanniohyton magnificum* Harms (Rubiaceae) contain schumanniofidoside, a chrome alkaloid glycoside (Akunyili and Akube, 1986). It reduced the lethal effect of Cobra (*Naja melanoleuca* Hall) in albino mice. Rhizomes of *Mandevilla velutina glabra*
are widely used against snakebite in Brazil. The ethanolic extract possesses antibradykinin action in isolated rat uterus against *Bothrops jararaca* venom (Calixto *et al.*, 1985). Methanolic extract of the stem and root of *Schumanniophyton magnificum* (Rubiaceae) was reported to reduce the effect of Cobra venom cardiotoxin in chick biventer cervicis preparation (Houghton and Harvey, 1989). Mors *et al.*, (1989) reported on the in vitro neutralization of lethal and myotoxic activities of South American rattlesnake venom by the ethanolic extract of the aerial part of *Eclipta prostrata* L. (Asteraceae). Wadelolactone, sitosterol and stigmasterol were isolated from the extract which effectively neutralized lethal action of *Crotalus durissus terrificus* snake venom. Numerous plants which included extract of *Phyllanthus klotzschianu* (Euphorbiaceae), *Casearia sylvestris* (Flacourtiaceae) and *Apoleia leiocarpa* (Leguminosae) conferred 100% protection unto 48 hours after administration. Ferriera *et al.*, (1992) reported that the Ar-turmerone isolated from *Curcuma longa* (Zingiberaceae) inhibits haemorrhagic activity of *Bothrops jararaca* venom and the lethal effect of *Crotalus durissus terrificus* in mice.

Ehretianone, a new quinonoid xanthene was isolated from the methanolic root extract of the root bark of *Ehretia buxifolia* had antisnake venom activity against Echis carinatus venom in mice (Selvanayagam *et al.*, 1996). The root bark of *Tabernaemontana catharinsis* (‘leiteiro’, ‘cow milk’) is believed to neutralize the effect of the *Crotalus durissus terrificus* venom. The ability of the lyophilized aqueous extract and of a pure compound obtained from the ethanolic extract of *Tabernaemontana catharinsis* to inhibit the lethal and myotoxic activities of *Crotalus durissus terrificus* (South American rattlesnake) venom was reported. (Batina *et al.*, 2000). Rahmy and Hemmaid (2001), reported that oral administration of garlic could be used as a prophylactic tool for cobra snake envenomation. Extract of *Mucuna pruriens* (L) DC seeds have been reported to antagonize the coagulative cascade which is stimulated by *Echis carinatus* venom (Guerranti *et al.*, 2001). Aqueous extract from *Casearia sylvestris* leaves was able to neutralize the haemorrhagic activity caused by *Bothrops asper*, *Bothrops jararacussu*, *Bothrops moojenii*, *Bothrops neuweidi* and *Bothrops pirajai* venoms (Borges *et al.*, 2001). PLA₃ enzymes are related with a wide variety of pharmacological activities and among this is myotoxicity which was neutralized by *C. sylvestris* (Borges, 2000).
Plant extracts of *Bursera simaruba*, *Chusia torresii*, *C. palmana*, *Croton draco*, *Persea americana*, *Phoebe brenesii*, *Pimenta dioica*, *Sapindus saponaria*, *Smilax cuculmeca* and *Virola koschynyi* were found to inhibit hemorrhagic activity induced by the venom of the snake *Bothrops asper* (Castro *et al*., 1999). Chemical analysis of these extracts identified catequines, flavones, anthocyanines and condensed tannins, which may be responsible for the inhibitory effect observed, probably owing to the chelation of the zinc required for the catalytic activity of venom’s hemorrhagic metalloproteinases. In the coastal area of Papua New Guinea, various plants are used to treat bites of two snakes namely, the death adder, *Acanthophis* sp. and the small eyed snake, *Micropechis ikaheka*. The plants used were, *Alphitonia incanea*, *Cerbera floribunda*, *Mangifera minor*, *Machura sp*, *Melanolepis multiglandulosa*, and *Osmoxylon micranthum* (Mebs, 2000).

The present investigation:

In the present investigation, fifteen plants were selected to test for neutralization of snake venom induced lethal action. They are as follows:

*1. Andrographis paniculata* Nees (aerial parts)
*2. Aristolochia indica* Linn (root)
*3. Crotalaria juncea* Linn (flower)
*4. Croton tiglium* Linn (seeds)
*5. Dolichandrone spathacea* (L.f) k. Schum (seeds)
*6. Emblica officinalis* Gaertn (root)
*7. Hemidesmus indicus* R.Br (root)
*8. Pluchea indica* Less (root)
*9. Strychnos nux vomica* Linn (seed)
*10. Vitex negundo* Linn (root)
11. *Trichosanthes dioica* Roxb (root)
12. *Stephania japonica* (Thumb) (Miers) (root)
13. *Allium sativum* Linn (root)
14. *Curcuma longa* Linn (root)
15. *Moringa pterygosperma* Gaertn (stem/bark)
16. *Achyranthes aspera* Linn (aerial part)
17. *Curcuma zedoaria* (rhizome)
18. *Gloriosa superba* (root)
Out of the eighteen plants, ten plants (*) were found to be effective in snake venom neutralization. Three plants were selected which had the highest potency against snakebite. They are *Pluchea indica*, *Hemidesmus indicus* and *Strychnos nux vomica*.

*Pluchea indica* belongs to the family *Compositae*. It is a richly branched shrub with alternate pale green, dentate (toothed) leaves (Fig. 19). The terminal flowers are rose-purple in colour. This plant is a native of India, South China, Malaysia and the Pacific. It is believed to have a rich medicinal value. It reduces muscle pain, helps with haemorrhoids, helps in the treatment of kidney stones, lowers blood sugar, diuretic, reduces pain, promotes digestion and is an elixir of longevity. The leaves and roots are believed to possess astringent and diaphoretic properties. Infusion of leaves are used in lumbago and leucorrhoea. In Malaysia, leaf juice is prescribed in cases of dysentry. The leaves are also used as nerve tonics (Nandkarni, 1976). Dhar (1973) reported that the whole plant including root has a gross effect on CNS. Sen et al (1993) has shown that root extract of this plant possesses antiinflammatory activity. The chloroform extract showed significant inhibitory activity against carrageenin-, histamine-, serotonin-, hyaluronidase- and sodium urate-induced pedal inflammation. The extract inhibited protein exudation and leucocyte migration. The extract also inhibited carrageenin- and cotton pellet-induced granuloma formation as well as turpentine induced joint edema and adjuvant-induced polyarthritis. (Sen *et al.*, 1991). The root extract is also found to exert neuropharmacological actions in socially isolated mice. Locomotor activity was higher on treatment with the extract and prolonged pentobarbital sleep induction in a dose dependant manner (Thongpraditchote *et al.*, 1996). Chemical compounds isolated from *P. indica* was first reported by Mukhopadhyaya (1983). Presence of 3-(2'3'-diacetoxy -2' methyl butyryl) cuauahemone was indicated. Uchiyama (1989), reported the presence of five new triterpenoid glycosides, linaloyl glucoside, linaloyl apiosyl glucosyl, 9-hydroxyl linaloyl glucoside, plucheoside A and B. Uchiyama *et al.*, (1991) showed the presence of seven new compounds out of which four are triterpenoids and three are lignin glycosides, from the methanolic fraction of the root.

(68)
**Hemidesmus indicus** belongs to the family *Asclepiadaceae* (Fig. 20). Its root is widely used as a demulcent, diaphoretic, diuretic, tonic. The plant is abundant in India. Its habitat is mainly upper gangetic plain, eastwards to Bengal and the Sundarbans, and from the Madhya Pradesh to south India. *Hemidesmus indicus* is studied widely for its chemical constituents and medicinal values. Phytochemical studies on the roots of *Hemidesmus indicus* resulted in the isolation of six new pentacyclic triterpenes including two oleanenes, three ursenes and a lupene including, beta-amyrin acetate (Roy *et al.*, 2001). Two novel pregnane glycosides, denicunine (1) and heminine (4), have been isolated from the dried stem of *Hemidesmus indicus* R.Br. (Sigler *et al.*, 2000). Numerous studies were carried out on the medicinal value of this plant. Oral treatment with the ethanol extract of *Hemidesmus indicus* roots (100 mg/kg, for 15 days) significantly prevented rifampicin and isoniazid-induced hepatotoxicity in rats (Prabakan, 2000). Gupta, in his study has shown the antileprotic action of the root extract in experimental mice (Gupta, 1981).

**Strychnos nux vomica** is one of the most popular medicinal plants of this subcontinent. It is widely prescribed by the practitioners of homeopathic medicine. It belongs to the family *Strychnaceae*. It is a medium-sized tree irregular branches, flowers small, greeny-white, funnel shape (Fig. 21). Fruit about the size of a large apple with a smooth hard rind or shell which when ripe is a lovely orange colour, filled with a soft white jelly-like pulp containing five seeds. The seeds are removed when ripe. They have the shape of flattened disks densely covered with closely appressed satiny hairs, radiating from the centre of the flattened sides and giving to the seeds a characteristic sheen; they are very hard, with a dark grey horny endosperm in which the small embryo is embedded; no odour but a very bitter taste (Fig. 22). Nux Vomica contains the alkaloids, strychnine, brucine, also traces of a glucoside Loganin, about 3 per cent fatty matter, caffeotannic acid and a trace of copper. The pulp of the fruit contains about 5 per cent of loganin together with the alkaloid strychnine. The seeds are known for its medicinal value in very low doses. Due to the presence of strychnine it is highly poisonous but useful in low doses. High dilution doses have been studied in experimental rats and was found to reduce...
Fig 19: *Pluchea indica* Less

Fig 20: *Hemidesmus indicus* R.Br.
Fig 21: *Strychnos nux vomica* Linn

Fig 22: Seeds of *Strychnos nux vomica*
voluntary ethanol intake (Sukul et al., 2001). They are used in atonic dyspepsia, stimulant on the gastro-intestinal tract. It stimulates peristalsis, in chronic constipation. It is also believed to improve the pulse and raises blood pressure and is of great value as a tonic to the circulatory system in cardiac failure. In cases of surgical shock and cardiac failure large doses are given up to 1/10 grain by hypodermic injection; also used as an antidote in poisoning by chloral or chloroform. Studies with the root extract showed antidiarrhoeal activity (Shoba and Thomas, 2001). It has been investigated that strychnine, the major active principle in the alcoholic extract of the seeds of Strychnos nux vomica, is responsible for its antilipid peroxidative property. The mechanism of action of this drug is through the chelation of the free iron in the system. It has also been observed that strychnine does not have any pro oxidant property, because it does not convert Fe$^{3+}$ to Fe$^{2+}$ and vice versa in the reaction system, as has been observed with several other antioxidants (Tripathi and Chaurasia, 2000). Crude alkaloid fraction of the nux vomica seeds were found to possess antinociceptive effects in mice (Cai et al., 1994). Numerous polysacharrides mainly galactomannans have been isolated from the seeds which are found along with the alkaloids (Corsaro, 1995). Thirteen alkaloids were isolated from the seeds of Strychnos nux vomica. They were identified as strychnine, beta-colubrine, pseudostrychnine, strychnine N-oxide, brucine, brucine N-oxide, novacine, icajine, vomicine, isostrychnine, isobrucine, isobrucine N-oxide and isostrychnine N-oxide by chemical and spectroscopic analysis (Yang and Yan, 1993).