Chapter 7

DISCUSSION
Snakes are one of the most feared animals on this planet. Evolution theories suggest that snakes, which belong to the group reptilia, have always been powerful than the other members of the animal kingdom. As a result, snakes have inherited the capacity to threaten other animals and cause intense fear among them. Due to this fear, snakes are worshipped and treated as a symbol of power and strength in almost every culture and civilization on earth. This obsession to respect and fear for snakes has been generated over the ages due to their capacity to produce and store venom.

Lack of proper knowledge about snakes often leads to an enormous proportion of fear and misconception. Not all snakes are venomous. Less than 20% of the total snake population are venomous (Warrel, 1987). Snakes are in general, shy and timid by nature and avoid human company. It is widely believed that snakes go out of their way and chase humans. On the contrary, unless threatened snakes do not attack humans. Exceptions being, the female King Cobra chase its enemies while guarding her eggs. The Black Mamba known for its nervousness chases the enemy when threatened. The Saw scaled viper is an aggressive snake in defence. Even these snakes prefer to escape rather than attack. We must understand they are just another animal whose only need is to survive long enough to reproduce.

The fear of being attacked and killed by the snake, has forced humans, resort to extreme measures in the name of defence and survival. It has resulted in the slaughtering of huge population of harmless, beneficial and eco-friendly snakes. Fayrer, in 1890, estimated that 510,659 snakes were killed in India at a cost of Rupees 19,004 and in 1892, 578,415 snakes were destroyed at a cost of Rupees 23,556 !! (Hagwood, 1996). Sacrifice in such great numbers of one particular species, in one region, disturbs the ecological balance of that area and often make that species endangered or even extinct. At that time, there
was no scientific cure to snakebites. Hence the only solution was to eliminate the animal itself.

Snakes are beneficial to humans. They are carnivorous and feed on the destructive animals like rats and mice. Rats and mice are destructive because they carry disease and destroys crops and grains. Snakes therefore act as pest controllers because of their extensive predation on rats and mice and keeping a check on their population. Certain snakes, like the brown snake feed on earthworms, insects, spiders, small fish and small frogs. Thus they form an important link in the food chain and maintain ecological balance. Snake venom has numerous medicinal values and is also used for preparing commercial snake venom antiserum, the only treatment for snakebite.

The beneficial effects of the snakes in our lives is largely overshadowed by the extreme fear and dislike for snakes. The venom that they possess is the main cause of this. The presence of venom is an evolutionary adaptation to immobilize its prey and is used secondarily to defend and kill. Snake venom is a highly toxic secretion of the venomous snakes. It is stored in specialized gland called venom glands and is delivered by the two fangs.

Snake venom has been the cause of innumerable deaths worldwide. Thousands of people die every year in India alone. Hati et al. (1992) estimated 10,000-15,000 deaths per annum in India. India is a snake infested country. The Indian states with highest snakebite incidences are West Bengal, Tamil Nadu, Uttar Pradesh and Kerala (Gaitonde et al., 1980). The snakes responsible for such high mortality rate are King Cobra, Saw scaled viper, Russell’s viper and Banded Krait. These snakes are abundant in the districts of Midnapur, Birbhum, Bankura, 24 Parganas and Hooghly of the Indian state of West Bengal as shown in the map (Fig 7.1).

The villagers, specially the farmers fall prey to the deadly venom while at work. The odd encounter with a snake specially at night is an occupational hazard for farmers in most of the tropical countries including India. Often it is found that the public health centres are far from the site of accident or that these centres lack any medical facility required for these snakebite victims. The patients finally reach the large district hospitals when it is already too late.
Fig. 7.1 Geographical distribution of venomous snakes in West Bengal, India
Since the last Century, snake venom antiserum developed by Calmette in 1894, is the only treatment for snakebite. Though a viable treatment, it has many drawbacks. It does not provide enough protection against various snake venom induced actions like haemorrhage, necrosis and nephrotoxicity. Snake venom antiserum requires ideal storage conditions which are generally not met with, specially in the villages and often they are not easily available to the villager. To add to their woes, the snake venom antiserum is too costly for them to afford. As a consequence, the poor villager takes the help of local medicine men or ojhas, snake stones and medicinal plants. The traditional healers who are abundant in the rural areas of India, prescribe various concoctions of medicinal plant parts which are either applied on the wound or taken orally. These traditional healers are very popular in their communities due to two reasons. Firstly, not all snakebites are fatal. It might be possible that the snake was not venomous or maybe not enough venom to kill the patient, was injected. Secondly, with a strong heritage of the use of Indian medicinal plants to treat numerous diseases, the combination of plant extracts may actually have been life saving.

Regardless of which mode of treatment, the victim goes for, the mortality rate due to snakebite could not be controlled even in the 21st Century. Since 1894, antiserum has been the only treatment and an alternative possible cure is yet to be clinically established. With increasing scientific awareness on animal protection among the people, doubts are being raised about the procedure of raising the snake venom antiserum in horses, that was developed by Calmette. The procedure involves injecting low doses of snake venom to horses for a prolonged time schedule. Following this, the antibody against the snake venom is obtained from the serum of the horses blood. Recently, on the basis of newspaper reports (The Statesman, dated 15.02.02), it was known that the Supreme Court of India has given a verdict on the issue of ill treatment of horses in the serum manufacturing institutes of India, considering a ban on antiserum production. It may be a possibility then, that in distant future the snakebite victims will be left without any specific treatment to save their lives.
The scientific community, specially in the regions with high incidence of snakebite are forced to turn their attention towards exploring alternatives to snake venom antiserum. The alternative therapeutic management can be obtained from nature itself. The use of natural products with therapeutic properties is as ancient as the human civilization and, for a long time, plant and animal products were the main sources of drugs (De Pasquale, 1984). Medicinal plants with its enormous potential are gradually explored for utilizing them for specific therapeutic management. Plants are extensively used as drugs and its popularity can be fathomed by the facts stated below:

- **25% OF DRUGS PRESCRIBED WORLDWIDE COME FROM PLANTS**
- **121 SUCH ACTIVE COMPOUNDS ARE CURRENTLY USED**
- **OUT OF THE 252 ESSENTIAL DRUGS CONSIDERED BY WORLD HEALTH ORGANIZATION (WHO), 11% ARE OF PLANT ORIGIN**
- **60% OF ANTI-TUMOUR AND ANTI-INFECTIOUS DRUGS USED OR UNDER CLINICAL TRIAL HAVE NATURAL ORIGIN**

(Rates, 2001)

Herbal medicine is undoubtedly the oldest form of medicine, with thousands of years of history behind it. The discovery of medicinal plants may have occurred in a number of ways. Prehistoric people may have found therapeutic principles by trial and error, or perhaps by watching animals "treat" themselves by eating special plants when ill. In fact, “zoopharmacognosy” (the study of animal search of certain herbs to treat disease) has revealed that instinct consistently provides certain animals with therapeutic “information,” allowing them to use this natural system of medicine themselves. Because herbal therapeutics have proven efficacious by the standards of both history and modern medicine, plant medicines are the subject of close scrutiny by major drug companies. A medicinal plant can be any plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process or any plant that is employed as a source of drugs or their precursors (Arias, 1999). Conventional doctors might argue that a single active constituent may be more precisely characterized, and that “extraneous” chemicals contained within the
whole plant complicate their understanding of its action. Holistic doctors believe that prescriptions of whole plants provide these advantages: (1) synergistic action and (2) safety. The entire world has started paying their attention to the vast resource available in nature for the benefit of mankind.

India has a rich tradition of the usage of medicinal plants. Therapeutic uses of plants have been scripted in ancient epics like Mahabhatrata and Ramayana. For the last few centuries, numerous records have been discovered where the use of traditional medicine is mentioned. These knowledge were systematically recorded and incorporated into regular system of medicine that refined and developed and became a part of the Materia Medica of these countries. The ancient civilization of India and also other ancient civilizations like China, Greece, Arab and Africa, developed their systems of medicine like Ayurveda, Unani, Homoeopathy which are independent of each other but all of them were predominantly plant based. From history we learn that in the ancient times, India was known as a place of rich natural resources, knowledge, wisdom and scholarship. People from other countries of the world as China, Cambodia, Indonesia and Baghdad used to come to the ancient universities of India like Takshila (700 BC) and Nalanda (500 BC) to learn health sciences of India, particularly ‘Ayurveda’. It is perhaps the oldest (6000 BC) among the organized traditional medicine. It has gone through several stages of development in its long history. It spread with Vedic, Hindu and the Buddhist cultures and reached as far as Indonesia in the east and to the west it influenced the ancient Greek who developed a similar form of medicine.

The various alternative systems of medicine in India functions through two social streams. The folk stream comprising mostly the oral traditions practiced by the rural villages. The carriers of these traditions are millions of rural folk who still continue the tradition. The other is the classical or the scientific stream of medicine. This comprises of the codified and organized medicinal wisdom with sophisticated theoretical foundations and philosophical explanations expressed in classical texts like ‘Charka Samhita’, ‘Sushruta samhita’, ‘Bhela samhita’, and hundreds of other treatises including some in the regional languages covering treaties of all branches of medicine and surgery. Systems like Ayurveda, Siddha, Unani, Amchi and Tibetan etc are expressions of the same.
Today there is a renewed interest in traditional medicine. During the past decade there have been an ever increasing demand especially from developed countries for more and more drugs from plant sources. This revival of interest in plant derived drugs is mainly due to the current widespread belief that ‘green medicine’ is safe and more dependable than the costly synthetic drug, many of which have adverse side effects. This resurgence of interest in the plant based drugs have necessitated an increased demand of medicinal plants leading to over-exploitation, unsustainable harvesting and finally to the virtual decimation of several valuable plant species in the wild. Moreover, the habitat degradation due to increased human activities (human settlements, agriculture and other developmental programmes), illegal trade in rare and endangered medicinal plants and loss of regeneration potential of the degraded forests have further accelerated the current rate of extinction of plants particularly the medicinal plants. Medicinal plants are potential renewable natural resources. Therefore, the conservation and sustainable utilization of medicinal plants involving a long term, integrated, scientifically oriented action programme is necessary. This includes aspects of protection, preservation, maintenance, exploitation, conservation and sustainable utilization. A holistic and systematic approach envisaging interaction between social, economic and ecological systems will be a more desirable one. The most widely accepted scientific technologies of biodiversity conservation are the in-situ and ex-situ methods. Though India has a rich biodiversity, the growing demand is putting a heavy strain on the existing resources. While the demand for medicinal plants is growing, some of them are increasingly being threatened in their natural habitat. For meeting the future needs, cultivation of medicinal plant has to be encouraged. According to an all India ethnobiological survey carried out by the Ministry of Environment & Forests, Government of India, there are over 8000 species of plants being used by the people of India. Worldwide there has been a huge growth in the popularity of alternative medicine and herbal products mainly because they do not have harmful side effects when genuine medicinal plants are used. To convert the potential of our medicinal plants into economic wealth, the medicinal species can be categorized into: (a) those which are of proven medicinal value as per scientific parameters, (b) those on which sufficient leads are available, and (c)
- **ATIS** *Aconitum heterophyllum* Wall Used as antipyretic, astringent, tonic. Used in diarrhoea, indigestion, cough, troubles during dentition in children.

- **BAEL** *Aegle marmelos* L.Corr. Used as an astringent, carminative, cooling agent, laxative, also used in colitis, diarrhoea, dysentery and flatulence.

- **BRAHMI** *Bacopa monnieri* L. Used as nervine tonic/memory enhancer.

- **CHANDAN** *Santalum album* Linn Used as antiseptic and a cooling agent. The wood round up with water into a fine parts is commonly applied to local inflammations, to the temples in fever and to skin diseases to allay heat.

- **GILOE** *Tinospora cordifolia* Wild Miers, ex Hook Used as rejuvenator, astringent, antipyretic, blood purifier and curative of dermatosis, pyrexia, skin diseases, gout, rheumatic and arthrites.

- **ISABGOL** *Plantago Ovata* Forsk Used as diuretic, used in inflammatory conditions of the mucous membrane of gastro intestinal and genitourinary tracts, in chronic dysentery, diarrhoea and constipation.

- **JATAMANSI** *Nardostachys jatamansi* De Used as tonic, antispasmodic, stimulant, antiseptic, diuretic, used in epilepsy, hysteria, chorea, convulsions, palpitation of heart, mental disorders, insomnia.

- **KALMEGH** *Andrographis paniculata* Nees Used in ayurvedic formulations for chronic malaria, jaundice, anemia and loss of apetite. Andrographis preparations are extensively used in different potencies for homeopathic medicines.

- **LONG PEPPER** *Piper longum* Linn Used as a tonic, in cough, cold, chronic bronchitis, palsy, gout, rheumatism, lumbago, insomnia, epilepsy, asthma, amoxeria, piles, dyspepsia, leucoderma etc.

- **MADHUNASHINI** *Gymnema Sylvestre* R. Br Used as astringent, tonic, refrigerant and antidiabetic. Leaves have a peculiar property of neutralizing temporarily the taste sensation for sugar and used in diabeti's. Also used in diabetes, liver disorders, cough and asthma.

- **SATAVARI** *Asparagus racemosus* Wild Used as antidiarrhoetic, refrigerant, antidysenteric, diuretic, demulcent, nutritive tonic, aphrodisiac and antispasmodic. Also used in consumption, epilepsy, diarrhoea, blood dysentery, haemophilic disorders and swellings.
Snakebite is a major sociomedical problem in a snake infested country like India. Since time immemorial, the people have tried all possible methods to combat this problem. They include snake stones, chanting mantras and also various herbal antidotes. Many plants are recommended in the traditional literature whose either root, seed or whole plant are widely used to treat snakebite. There are a few scientific reports about plants that neutralized snake venom but so far no scientific remedy has been formulated and clinically used.

Few medicinal plants having antisnake venom action are:

- *Andrographis paniculata* (Nazimuddin et al., 1978)
- *Diodia scandens* (Mittal et al., 1981)
- *Schumanniophyton magnificum* (Houghton and Harvey, 1988)
- *Eclipta prostrata* (Mors et al., 1989)
- *Curcuma longa* (Farreira et al., 1992)
- *Aristolochia indica* (Sadarsnan and Sivaprasad, 1995)
- *Gymnema sylvestre* (Sadarsnan and Sivaprasad, 1995)
- *Moringa oleifera* (Sadarsnan and Sivaprasad, 1995)
- *Hemidesmus indicus* (Alam et al., 1996)
- *Casearia sylvestre* (Borges et al., 2000)
- *Tabernaemontana catharinensis* (Batina et al., 2000)
- *Macuna pruriens* (Rahmy and Hemmaid, 2001)

Earlier in this laboratory, Alam et al., (1996) identified certain Indian medicinal plants with snake venom neutralizing property and a pure compound was identified from *Hemidesmus indicus* root extract Alam et al., (1994). The present study extends the search of evaluating selected Indian medicinal plants that are capable of snake venom antagonism. It is an effort to validate the claims made by tribal healers and medicine men and assess the activity of selected plants to treat snake envenomation in experimental animals. The study begins with the selection procedure of the Indian medicinal plants that would be used. Selection strategy is a decisive step and is based on i) information obtained from folk medicine of different cultures ii) common chemical composition of different plants or iii) random sampling and testing for medicinal value.

Exhaustive search for selecting the medicinal plants were carried out through literature survey and local tours to snake infested districts of West...
Bengal, India namely Midnapur, North and South 24 Parganas, Bankura, Birbhum and Hooghly. Information was gathered from the local healers, ojhas and villagers and samples were collected from them. Occasional non coopeation to reveal the exact combination of plant extracts prescribed by these medicinemen, was one of the few setbacks. The samples that were collected were sent to a team of plant taxonomists, Prof. N.Paria, University of Calcutta and Dr. A. Mukherjee, Burdwan University, India for authentication of the sample. Help was also received from one of the oldest organizations in India, the Botanical Survey of India, Shibpur, West Bengal. Another setback in the process of identification through local tours and literature survey was that the same plant had many local names varying from place to place or the same local name was coined for two or three plants.

Based on the local availability of the medicinal plants that were identified, a final list of eighteen Indian medicinal plants was prepared to test for antisnake venom properties in experimental animals. In India, the state of West Bengal is a snake infested region. The maximum number of deaths occur due to two snakes namely viper and cobra. Thus to test the activity of the plants, two venoms *Vipera russelli* and *Naja kaouthia* were used in this study.

Following the critical selection procedure, the selected plants were collected in bulk amounts from the local and reliable supplier M/s United Chemicals and Allied Products, Calcutta. It was ensured that the supplier obtained the plant materials from areas where the medicinal plants are cultivated for research and drug manufacturing purposes, though exceptions may have occurred. The date and place of the collection of plants were noted down since the medicinal property of plants do have geographical variations and potency gradually reduces with time.

The plant materials were stabilized by drying the material at ambient temperature in a shady place. It can also be carried out in an oven with controlled airflow and temperature. The dried plant materials were then powdered and taken for extract preparation. Since the basic chemical composition of the plants were unknown, the method of extract preparation was based on how the plant is used in folk medicine. Generally, aqueous extract of the plant part/whole
plant were used by making a paste in stone slabs (mortar and pestle), mostly applied over the wound or given orally in combination with other substances like black pepper, jaggery, lime, salt, honey etc, to exert its therapeutic effect. Based on these informations, aqueous extracts, methanolic and ethanolic extract of the plants were prepared in the laboratory. Extract preparation was carried out in Soxhlet apparatus by passing petroleum-ether for defatting, followed by a high polar solvent (methanol/ethanol). The extract was dried and stored in vacuo at room temperature till further use.

The critical steps that were involved at the beginning of the investigation can be summarized below:

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The dried plant extracts were dissolved in 0.9% saline and tested for snake venom neutralization in experimental animals. Solubility of each extract were noted. The plant extracts were highly soluble in water (60-85%), methanol (70-90%) and less soluble in chloroform (30-40%), benzene and petroleum-ether. The yield of the extracts obtained, varied from plant to plant (5-15%). It was expressed in terms of dry weight. The toxicity of the plant extracts were checked and snake venom neutralization studies were carried out with their non-toxic dose. Out of the eighteen plant materials selected for testing, ten plants were found to be active in neutralizing snake venom induced lethal action in experimental animals. They are:

(138)
- Seed extract of *Croton tiglium* Linn, *Dolichandrone spathacea* (L.f) k. Schum and *Strychnos nux vomica* Linn.
- Aerial parts of *Andrographis paniculata* and
- Flower extract of *Crotalaria juncea*.

In the present investigation, out of the ten plant materials, three of them, the root extracts of *Pluchea indica*, *Hemidesmus indicus* and seed of *Strychnos nux vomica* were found to possess highest snake venom neutralizing capacity against viper and cobra venom induced lethal action. These three extracts were therefore taken for a detailed study.

The basic procedure that is generally carried out when studying the efficacy of medicinal plants against any pathological condition can be seen at a glance below:

1. Fractionation
2. Purification

![Diagram]

1. Fractionation
2. Purification

(Rates, 2001)
The present study followed this basic principle while studying the snake venom neutralization with the three plant materials. For the sake of convenience, the studies with the three plants are discussed separately in three sections.

**Discussion on *Pluchea indica* root extract**

During the local tours to the districts of Midnapur and Hooghly it was found that the villagers used the plant *Pluchea indica* for fencing their huts. Further probe revealed that the area was infested with venomous snakes and such fencing provided a first line of defence for them. The local healers and ojhas included the root and whole plant extract of *Pluchea indica* in their magic mixture to treat snakebite victims. With such an information at hand, the methanolic root extract was tested against snake venom induced lethal action. From this laboratory, an earlier study was done on the snake venom induced lethal action neutralization by the root extract of *Pluchea indica* (Alam, *et al.*, 1996). Due to the lack of enough plant material, isolation and purification of the active constituents was not done at that stage. The present study was a continuation of the earlier study, with a view to identify the active constituents of *Pluchea indica* root extract.

The Indian medicinal plant *Pluchea indica* has been under scrutiny for its medicinal values for a long time. Reports of its anti-inflammatory and antipyretic action were reported by Sen *et al.*, (1993). The root extract was also found to inhibit carrageenan and cotton pellet induced granuloma as well as turpentine induced joint edema (Sen *et al.*, 1991). The extract also prolonged pentobarbitone induced sleep (Thongpraditchote *et al.*, 1996). Thiophene derivatives from *Pluchea indica* have been isolated from its root extract (Chakravarty and Mukhopadhyay, 1994). Two pentacyclic triterpenoids of rare occurrence have been isolated which possessed anti inflammatory activity.

These informations validates the claim of traditional literature, where the root extract was believed to have an effect on the nervous system and was used as an anti inflammatory agent. In the present study, methanolic root extract of *Pluchea indica* was found to induce a fall in body temperature, prolong pentobarbitone induced sleeping time as well as reduce mice paw edema. This
was in agreement to the previous reports. Snake venom envenomation by *Vipera russelli* and *Naja kaouthia*, induced several pathophysiological changes such as haemorrhage, inflammation, cardiotoxicity or neurotoxicity depending on the nature of the venom. Therefore crude extract was expected to possibly have the capacity to neutralize some of the pathophysiological changes caused by snake envenomation. In this study, the methanolic crude extract was found to neutralize viper venom induced lethal, haemorrhagic and defibrinogenating action and cobra venom induced lethal, cardiotoxic and neurotoxic activity. This observation suggested that root extract of *Pluchea indica* was an extremely potent antagonist against both viper and cobra venom.

Search for the active constituent responsible for snake venom neutralization was undertaken. The methanolic root extract was fractionated over silica gel chromatography. The fractions were eluted using solvent systems petroleum-ether, chloroform and methanol in increasing proportions. Pure compound was eluted by petroleum-ether : chloroform (40 : 60, v/v) and it was designated as PINF1. Another active fraction (PINF2) was eluted by chloroform. This fraction was further rechromatographed and pure compound was eluted by petroleum-ether : chloroform (70 : 30, v/v). The homogeneity of the compounds were tested by thin layer chromatography. Crystallization of the pure compound was carried out. PINF1 was found to be soluble in alcohol/methanol but insoluble in water. Spectral studies of PINF1 reveal that it is a long chain compound and PINF2 reveal that it is a mixture of β-sitosterol and stigmasterol. Both the compounds were taken for detailed snake venom neutralization study in experimental animals.

Spectral studies of PINF1 indicated that it was a mixture of long chain compounds. Due to the very low yield and also difficulty in fractionating the compound into a single compound, neutralization studies were carried out with the mixture. It is possible that a single compound, if isolated may not exert its snake venom neutralizing capacity in experimental animals. It was therefore decided to continue the pharmacological and neutralization studies with the mixture. PINF1 did not produce any lethal effect upto a dose of 25mg/kg body weight. It did not cause any change in the body temperature and pentobarbitone
induced sleeping time in mice, indicating it to be a safe and neutral compound. The neutralization studies showed that PINF1 was a potent compound against viper venom induced lethal, haemorrhagic, defibrinogenation and PLA2 enzyme activity. Cobra venom induced lethal action, cardiotoxicity and neurotoxicity studied in experimental models were also neutralized suggesting a highly potent snake venom antidote. It is well known that most of the herbal concoctions are not single compounds but used as mixtures, as it is believed that, in the mixture form the therapeutic capacity is exerted more effectively in synergy with others than a single compound. Therefore it is possible to consider the mixture for detailed analysis as a snake venom antidote. On the contrary, in order to satisfy mainstream doctors it would be desirable to identify the single compound responsible for neutralizing the snake venoms. Further work need to be carried out in this regard.

Spectral studies of PINF2 identified it as a mixture of β sitosterol and stigmasterol. β sitosterol is one of several plant sterols (cholesterol is the main animal sterol) found in almost all plants. High levels are found in rice bran, wheat germ, corn oils, and soybeans. It is a very common plant sterol and found in abundance. β sitosterol alone and in combination with similar plant sterols, reduces blood levels of cholesterol (Lees et al., 1977 and Pelletier et al., 1995). This appears to be because β sitosterol blocks absorption of cholesterol (Grundy et al., 1969). It has also been effective in reducing symptoms of benign prostatic hyperplasia (Berges et al., 1995). Although molecules quite similar to β sitosterol inhibit cancer cells in test tubes, the relevance of this information for people remains unknown (Kiriakdis et al., 1997). β sitosterol inhibits the action of the enzyme 5-alpha-reductase, which converts testosterone into DHT. DHT is one of the main contributing factors to benign prostatic hyperplasia. β sitosterol also inhibits the enzyme aromatase, which facilitates the production of estradiol. This is considered to be another important factor in the development of a benign prostatic hyperplasia. Anti-inflammatory, analgesic and anti pyretic activity of β sitosterol was reported earlier by Gupta et al., 1980. They also suggested a high margin of safety suggesting its application to human as medicine. In the present study, β sitosterol was found to induce a significant fall in body temperature and inhibit carrageenan induced
edema. In another study antihyperglycemic and insulin releasing effects of β sitosterol was observed (Ivorra et al., 1988). The anti-hyperglycemic effect of the sterol and sterolins are thought to be due to an increase in circulating insulin levels which is attributable to a stimulation of insulin secretion from pancreatic B-cells. These results indicate a possible anti-hyperglycemic use for the phytosterols in the prevention and treatment of pre-diabetic and diabetic conditions. Preliminary studies indicates that the plant sterols like β sitosterol may have a positive role to play in the complementary treatment of immuno-compromised patients. There may be a relationship between rise of CD4 lymphocytes and eosinophils. Plant sterol/sterolins have been demonstrated elsewhere to selectively increase CD4 lymphocyte counts (Donald et al., 1997).

In the present study, isolated β sitosterol was taken for assessing the snake venom neutralization against viper and cobra venom induced action. Earlier, Melo et al., (1988) isolated and purified wadelolactone, stigmasterol and sitosterol from Eclipta prostrata extract. These compounds were reported to inhibit snake venom induced haemorrhagic and myotoxic activity. In another study, the use of plant sterol like sitosterol to antagonize venom induced action was reported (Pareira et al., 1994). The mechanism of venom inhibition was not clearly understood. Not one particular mechanism of action can be singled out for the fact that β sitosterol was found to significantly antagonize both viper and cobra induced action. Snake venom antagonism by PINF1 and PINF2 against viper and cobra venom induced action can be compared in the figure below (Fig 7.2).

Viper venom as mentioned earlier affects the vascular system causing haemorrhage and defibrinogenation action whereas cobra venom is neurotoxic by nature. Snake venom envenomation causes a rise in serum phosphatase and transaminase levels. It is believed to be due to the lysosomal cell lysis thus changing the enzyme concentrations (Rainsford,1984). The lipoproteins of the lysosomal membranes may have been hydrolized since snake venom induces a rise in phospholipase enzyme concentration, thus causing cell lysis. β sitosterol prevented the venom induced changes in phosphatase and transaminase levels thus suggesting that maybe it has potent membrane stabilizing activity. To
Fig. 7.2 Comparison of PINF1 and PINF2 isolated from *Pluchea indica* root extract.

* No protection
confirm this, membrane stabilizing experimental models need to be carried out.

Although oxygen is central to life, the body’s utilization of this gas also results in the production of the undesirable metabolic by-product superoxide, a toxic free radical that can damage cellular components. In normal circumstances, the natural superoxide dismutase (SOD) enzymes catalytically remove superoxide by converting it into oxygen and hydrogen peroxide, which is quickly decomposed by another abundant enzyme, catalase. In its defence against pathogens, our immune system generates superoxide, which is converted first to hydrogen peroxide and then to hypochlorite, the active ingredient in chlorine bleach. Hypochlorite is the body’s chemical antiseptic. Superoxide dismutase is, therefore, an essential component in converting superoxide to the antibacterial agent, hypochlorite. In disease states such as inflammation, the immune system produces an excess of superoxide, overwhelming the native SOD enzymes’ ability to remove superoxide free radicals and leading to free radical induced damage to cells and tissues. Free-radical damage has been associated with a growing number of diseases and conditions, such as autoimmune diseases like rheumatoid arthritis, Parkinson’s disease, cancer, diabetic complications, stroke, myocardial infarction, pain, inflammation, injury during any trauma and also snake envenomation. It has already been mentioned earlier that PINF2 has anti-inflammatory and anti-pyretic activity. The present study observed that PINF2 antagonized the decrease of superoxide dismutase and also prevented lipid peroxidation caused by snake envenomation, thereby suggesting that the compound may stop free radical formation.

The ability of PINF2 to potentiate antiserum action against venom induced lethal action has been discussed in details later.

**Discussion on root extract of *Hemidemus indicus***

*Hemidemus indicus* R.Br or Indian sarsaparilla is a very common Indian medicinal plant. It is called ‘anantamul’ in the local dialect. *Sveta sariva* is the sanskrit name. It is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub. The plant is widely grown in the upper gangetic plains of
India and also from Madhya Pradesh to the south of India. The air-dried roots yield essential oil containing p-methoxy salicylic aldehyde as the major constituent. It is believed to act as a demulcent, diaphoretic, diuretic and blood purifier. It is employed in nutritional disorders, syphilis, gonorrhea, chronic rheumatism, gravel and other urinary diseases and skin infections.

This plant is a major component of the ayurvedic preparations as it is said to increase appetite and acts as a rejuvenator. *Hemidesmus indicus* promotes optimal growth of tissues and fights against strength-loss. *Hemidesmus indicus* is believed to possess antiviral effect. Babber, (1970) found antiviral activity in Sariva (*Hemidesmus indicus*) pharmacologically and it was suggested that this anti-viral activity may be due to the presence of interferon like factors in the plant. Antileprotic activity of indigenous drugs like “Anantamul” was for the first time tested on mice infected with *M. leprae*. The results were interesting and encouraging. It was reported to be a definite evidence in support of the drug causing a delay in multiplication of organisms in the mouse footpads (Gupta., 1981). Due to its immense medicinal importance, anantamul is found to be widely used as an alternative medicine. With even the WHO acknowledging the contributions of ethnomedicine in tackling several ailments, physicians are having a second look at alternative therapies all over the world. In this emerging scenario, the herbal treatment with various herbs including Indian sarsaparilla, is considered an important tool to tackle various pathophysiological conditions.

*Hemidesmus indicus* was found to possess antisnake venom action. Tours to the rural districts of West Bengal, India revealed that this aromatic root is ground to an aqueous paste and applied on the snakebite wound of the victim. It was also prescribed orally along with certain other compounds like salt, jaggery, lime etc. The rural folk had immense trust on this medicinal plant and preferred to cultivate the plant at home for their benefits.

Though many plants are used by rural healers or medicine men, very few of them have scientific evidence. However, *Hemidesmus indicus* have been studied earlier against snake venom neutralization in this laboratory. For the last twelve years, search for medicinal plants against snakebite has been the
endeavour in this laboratory. Under this programme, Alam et al., (1996) validated the claims of local medicine men that the root extract of *Hemidemus indicus* is a potent snake venom antagonist against viper venom induced actions. The methanolic crude extract was found to significantly neutralize viper venom induced lethal, haemorrhagic, necrotic, defibrinogenating, coagulant, fibrinolytic actions. A pure compound 2-hydroxy 4-methoxy benzoic acid was isolated and purified from the methanolic root extract and it was found to be a potent viper venom neutralizing compound (Alam et al., 1994).

The present study continued to explore *Hemidesmus indicus* as it was believed that there are more compounds present in the extract having greater snake venom neutralizing capacity. This was therefore an effort to validate once again, the claims of traditional literature of the usefulness of this plant in snakebite cases. The protocol of the study was similar to that done with *Pluchea indica*. The methanolic crude extract was prepared. It was highly soluble in water and less soluble in chloroform. This extract was non lethal upto 1.0gm/kg of experimental mice. The non lethal dose was taken for all the snake venom neutralizing study in experimental animals. The crude extract was found to significantly neutralize viper venom induced lethal, haemorrhagic and defibrinogenating action and cobra venom induced lethal, cardiotoxic and neurotoxic action.

The methanolic root extract was fractionated by column chromatography with solvents of increasing polarity. Each fraction eluted was dried and tested for venom neutralization in male albino mice. One fraction eluted with chloroform : methanol 95:05, v/v was found to possess antitsnake venom capacity. TLC pattern showed that it is not a single compound. Therefore further purification was carried out by rechromatography. Two active constituents were isolated. The first compound was obtained by eluting with chloroform : methanol 95.5 : 0.05, v/v. TLC showed that it had a single spot. Spectral analysis of the compound was not satisfactory. Variables like small yield, and constrains in purification might have prevented the complete purification to a single compound. It was therefore taken for neutralization studies in the form of a mixture. The fraction was designated as HINF1. Spectral study of HINF1 showed that it is a mixture of triterpenoids.
Despite the incomplete structure elucidation, HINF1 is considered an important finding due to its significant snake venom neutralizing effects. The compound was found to antagonize both viper and cobra venom induced action in both in vitro and in vivo studies in experimental animals. Mechanism of venom inhibition could be evaluated. It was found to neutralize the venom induced free radical formation as evaluated by thiobarbituric acid product (malonyldialdehyde) formation. It also inhibited venom induced SOD changes. HINF1 significantly potentiated antiserum action which will be discussed in detail later.

A second active constituent was isolated by rechromatography of the above fraction. It was eluted with chloroform : methanol 50 : 50. v/v. This component was designated as HINF2. TLC pattern indicated that the compound was a pure one. Spectral analysis of the compound confirmed it to be Lupeol acetate. Earlier studies have been done with the root of this plant. The roots were reported to contain a new ester identified as lupeol octacosanoate in addition to the known compounds viz., lupeol, -amyrin, lupeol acetate, -amyrin acetate, and hexatriacontane (Chatterjee and Bhattacharya, 1955). The present study further confirms the presence of lupeol acetate in the root extract. This is the first report of antivenom activity of lupeol acetate in experimental animals. Lupeol acetate is present in Aloe vera, Phyllanthus niruri (widely used as laxative and dysentery), Scaevola spinensis (used as antiviral, immunostimulant and possible anti tumour activity). The observation suggests that the plants that possess lupeol acetate might also have snake venom neutralizing capacity. It is therefore necessary to test those plants against venom induced activity in experimental animals. The alcohol extract of the bark of Alstonia scholaris a popular Indian medicinal plant contains lupeol acetate (Chopra., 1982). The extract have been shown to have anticancer activity against HS human sarcoma in chick embryo (Mukherjee et al., 1942). Some other compounds that are present includes two novel pregnane glycosides (C21), denicunine and heminine, isolated from the dried stem of Hemidesmus indicus R.Br (Sigler et al., 2000) and not root.

HINF2 or lupeol acetate was found to be a potent snake venom neutralizing factor. Viper and cobra venom induced action was significantly antagonized by
HINF2. Snake venom antagonism by HINF1 and HINF2 against viper and cobra venom induced action in experimental animals can be compared in the figure below (Fig 7.3). When compared with 2-hydroxy 4-methoxy benzoic acid, isolated and purified earlier (Alam et al., 1994) it was found that HINF2 was more potent against venom induced action. The latter was able to antagonize both viper and cobra venom induced action, whereas 2-hydroxy 4-methoxy benzoic acid was effective against viper venom only. HINF2 was found to potentiate antiserum action significantly.

The mechanism of action of venom inhibition was studied. As discussed earlier, venom induces changes in the level of transaminase and phosphatase activity. Lupeol acetate like the other compound neutralized the venom induced changes in transaminase and phosphatase levels. 2-hydroxy 4-methoxy benzoic acid is a derivative of salicylate and aspirin. It was observed that the compound exerted effects that mimics aspirin action. Recent studies report that aspirin treatment improves allergic diseases specially asthma (Perez et al., 2002) by suppressing various enzymatic activities. Much is not known about all the enzymes active in snake venom envenomation. Comparison of the activities of known compounds on enzymes which are also effective in snake venom envenomation might be helpful in developing a chemical snake venom antagonist. Aspirin is also a highly cost effective prophylactic in heart diseases (Elwood, 2001). Snakebite severely effects the cardiovascular system of the victim. Studies showing that a salicylate which is effective in heart disease is similar to another derivative which is a snake venom antagonist. More studies need to be conducted with the compounds isolated from Hemidesmus indicus on their mechanism of action since they are structurally similar to known effective medicines. The mechanism of action of lupeol acetate on other pathophysiological states need to be explored and then compare its inhibition on snake venom induced pathophysiological states.

The salicylate derivative isolated from Hemidesmus indicus had some disadvantages when compared to Lupeol acetate. It induced gastric damage by acting on the mucosal cells leading to capillary damage, acid secretion and H+ ion back diffusion causing gastric ulcers (Rainsford, 1984). Lupeol acetate on
Fig. 7.3 Comparison of HINF1 and HINF2 isolated from *Hemidesmus indicus* root extract.

* No protection
the other hand was devoid of any serious side effects suggesting that it would be useful as a snake venom antidote in clinical trials. The present study was an attempt to identify the nature of the compound responsible for anti snake venom action. The observation might satisfy the mainstream practitioners who raise doubts about the immense healing capacity of medicinal herbs.

**Discussion on seed extract of Strychnos nux vomica**

*Strychnos nux vomica* is one of the most popular medicinal plant used worldwide. Its uses are scripted in details in the *Materia medica* (Clarke, 1902). Hannelmann, the father of homeopathic medicine had mentioned the uses of *Strychnos nux vomica* in various pathophysiological conditions. The bark and seed were said to have antisnake venom activity. Mhaskar and Caius (1931) observed that the seed extract of *Strychnos nux vomica* was highly toxic and did not exhibit any anti snake venom activity. There has been no report of snake venom neutralization action by the seed extract of *Strychnos nux vomica*. The clue about the seed extract to antagonize venom was obtained from a local medicine man of Bankura district, West Bengal, India. Doubts were there since the seeds of *Strychnos nux vomica* are highly toxic themselves. The principle of homeopathy was studied where extreme dilution of a toxic compound highlights the medicinal value of that compound. On diluting the ethanolic extract to non lethal dose, the first attempt to study snake venom neutralization was done. Neutralization of venom induced action in experimental animals was observed quite unexpectedly. There was initial difficulty in standardizing the exact concentration when the extract is non lethal as well as capable of neutralizing snake venom. The crude extract in very low dilution (1 : 10,000 v/v) was found to significantly neutralize viper venom induced lethal, haemorrhage and defibrinogenation action and also cobra venom induced lethal, cardotoxic and neurotoxic action. *Strychnos nux vomica* seed extract is believed to be the prime natural source of strychnine and other alkaloids like brucine. The seeds of this plant has been under scrutiny for a long time. Recently, a capillary zone electrophoresis method was developed for the separation and determination of strychnine and brucine in *Strychnos nux-vomica* L. and its preparation (Chen et al., 2000). The recovery was 102.96% for strychnine and 98.56% for brucine.
Other parts of the plant have also been studied in detail. Six compounds were isolated from the root and stem of *Strychnos nitida* for the first time. On the basis of chemical properties and spectral data, the compounds were identified as β-sitosterol, strychnine, brucine, cantieyine, lignoceric acid and palmitic acid (Gu et al., 1997). Extraction, determination and identification of the alkaloids in differently processed products of the seeds of *Strychnos nux vomica* was done by Cai et al., (1994). Thirteen alkaloids have been isolated from the seeds of *Strychnos nux vomica*. They were identified as strychnine, β-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). Since, alkaloids constitute the major portion of the seed extract, it would be proper to assume that the snake venom antidote might be a compound, which is present in lower concentration in the seed.

Isolation and purification of the active constituent was done. Column chromatography of the extract was avoided since the extract had highly toxic strychnine and similar alkaloids in greater concentration. As a result it would be difficult to isolate the compound which was presumed to be present in very low amount, from the concentrated crude extract. To make things simpler, preparative TLC of the crude extract was done. Numerous bands were obtained. The known compound like strychnine were also taken for comparative TLC and the band containing strychnine in the TLC of crude extract was identified and removed. Each band was eluted in methanol, dried and then tested against venom induced lethal action. A single band was finally identified at $R_f$ 0.4 which showed venom inhibition. The band was separately taken for second TLC where three more bands were obtained. As done before, each band was taken for neutralization study and single compound was identified. It was eluted and filtered (millipore) and then further fractionated over column chromatography and pure compound was eluted by ethyl acetate : methanol : water 80 : 19 : 01 v/v. The compound was designated as SNVNF. Yield of SNVNF was found to be 0.04%. Spectral studies indicated that it is a very small compound. NMR spectra reveals the presence of only 3C suggesting that it has a very low MW and might be phenolic in nature. The exact structure could not be confirmed due to very small yield and difficulty in separation.
SNVNF was found to effectively neutralize both viper and cobra venom induced action. If compared to the earlier pure compounds isolated and purified from the root extracts of *Hemidesmus indicus* and *Pluchea indica*, it may be suggested that SNVNF, despite being a very small compound and structure yet not confirmed, it demonstrated very significant snake venom neutralizing capacity in experimental animals. It would be proper to mention that the dose of SNVNF taken for venom induced lethal action neutralization study was $10 \pm 0.77 \mu g/mice$. Snake venom neutralizing capacity at such low dose indicates the strong potency of the compound. Antiserum action against venom induced lethal action was also significantly potentiated, again confirming high potency of SNVNF.

The structure and function of SNVNF in snake venom neutralization need further investigation before it can be claimed as a snake venom antidote. This study for the first time reports the presence of such an active compound in the seed extract of *Strychnos nux vomica*. Since time immemorial, the medicinal values of the seeds of *Strychnos nux vomica* have been studied and utilized. Seeds of *Croton tiglium* has been mentioned in literature (Chopra et al., 1956) having snake venom neutralizing capacity. The present investigation had also confirmed the claim that *Croton tiglium* seed extract significantly neutralized snake venom induced lethal action in male albino mice. The present investigation also mentions the snake venom neutralizing capacity of the seed extract of *Dolichandrone spathacea*. It is a very common plant found in West Bengal, India. The seeds are present within the pod like fruit. Ethanolic seed extract was found to significantly antagonize viper venom induced lethal, haemorrhagic, defibrinogenating and cobra venom induced lethal, cardiotoxic and neurotoxic activity (result not shown). No active constituents have been isolated from these seed extracts as yet. Isolation and purification of SNVNF and its high level of potency focusses the need to study the other seeds in snake venom neutralization.

One of the common practices of traditional healers, medicine men, ojhas etc is that they generally prescribe the medicinal plants in combination and also add other substances like lime, jaggery, salt, honey etc. They believe that these combinations stimulate the therapeutic effect of the main drug (plant).
Scientific evidence on the efficacy of this claim is negligible. On comparing the compounds isolated from *Pluchea indica* and *Hemidesmus indicus*, it was found that the two compounds isolated from *Pluchea indica* are more potent against *Naja kaouthia* venom and those isolated from *Hemidesmus indicus* are more potent against *Vipera russelii* venom. In the present investigation, a minor study on the effect of combination of the pure compounds was done (results not shown). PINF1, PINF2, HINF1, HINF2 were used in combination to test the inhibition of snake venom induced lethal action. The results obtained were not very promising. Protection was almost similar to that of, what was found with the single compound. It can be thus suggested that the combination of pure compounds may not be as potent as combination of the crude extract as observed in the present study. Earlier, study with combination of crude extracts showed high degree of protection (result not shown). It is therefore possible that, compounds other than active constituent may be responsible in stimulating the neutralizing effect of the crude extract. Also, presence of more active constituents which are yet to be identified may be responsible for this increased potency. Further study are necessary in this regard.

**Discussion on antiserum action potentiation**

Since 1894, commercial snake venom antiserum developed by Calmette is the only specific treatment available for snakebite victims. In the present day, most commercial antivenom consists largely of F(ab)2 fragments of immunoglobulins obtained by pepsin digestion and ammonium sulphate precipitation (Chippaux and Goyffon, 1998). There are two types of antivenom found throughout the world, the polyspecific (developed against more than two snake venoms) and monospecific (developed against one specific snake venom).

In India, snake venom antiserum are prepared commercially in Bengal Chemical and Pharmaceutical Limited, Calcutta; Haffkine Biopharmaceutical Corporation Limited, Mumbai; Serum Institute India Limited, Pune and Central Drug Institute, Kasauli. They are the manufacturers of equine polyspecific antiserum against the venom of Indian snakes.
 Though a viable antidote, snake venom antiserum does not give enough protection against venom induced haemorrhage, necrosis and nephrotoxicity. (Corrigan, 1978; Stahel, 1985 and Sutherland, 1992). One of the most common difficulty faced after administration of the commercial antiserum to a snakebite patient is developing severe hypersensitivity reaction. This can be termed in general as serum sickness. Serum sickness results from a reaction to an antigen, a protein that the body recognizes as foreign. The classic example of a cause of serum sickness is an antiserum administered following a snakebite to counter the poisonous venom. Serum sickness will usually develop within 7 to 10 days after initial exposure to the antigen; at times, however, the reaction does not develop until as long as three weeks later. With subsequent exposures, serum sickness tends to develop more rapidly (within one to four days) and only a very small amount of the substance may cause an intense response. The first signs of serum sickness are redness and itching at the injection site. Other signs and symptoms include:

- Skin lesions, possibly including bruise-like patches from bleeding into the skin; a faint red discoloration over the hands, fingers, feet, and toes before other lesions or a brighter rash erupt; hives
- Joint pain
- Fever
- Malaise (feeling unwell)
- Swollen lymph nodes
- Swelling, especially around the face and neck
- Wheezing
- Flushing
- Runny nose
- Rarely, low blood pressure, as with anaphylaxis (a severe, total body allergic reaction)
- Muscle pain
- Diarrhoea, nausea, abdominal cramping

Case studies reported that late reaction of snake envenomation was serum sickness. The general symptoms of serum sickness were itching, urticaria, fever,

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arthralgia, proteinuria and neurotopical symptoms. For mild attacks, antihistamines were administered but with severe cases with neurological symptoms, a short course of prednilosone was given (Meier and White, 1995).

Antigens are proteins that stimulate the body to produce antibodies. These antibodies form complexes with the antigens and, in the case of serum sickness, become trapped on endothelial surfaces, layers of cells that line the heart, blood vessels, lymph vessels, and other body cavities. This leads to a series of immune-system reactions that cause the symptoms of serum sickness. A snakebite patient is more likely to suffer from serum sickness if high quantities of snake venom antiserum are required.

The hypersensitivity or serum sickness caused due to snake venom antiserum can be avoided if the patient is aware of his/her hypersensitivity to antiserum and if the healthcare provider can perform skin tests to check for serum sensitivity before giving antiserum. This is not possible to do in an emergency situation like snakebite. Therefore any method to desensitize to the antiserum, at least temporarily cannot be done by the health provider. To avoid severe complications despite antiserum administration to the patient, doctors typically prescribe antihistamines or analgesics for serum sickness. If symptoms do not respond to this treatment, they may prescribe corticosteroids, resort to plasmapheresis, a procedure for removing blood, separating plasma from the blood, then replacing the blood along with plasma substitutes. The condition of the patient deterriorates during this process.

Alternative and complementary therapy is necessary to prevent such conditions. Serum sickness due to snake venom antiserum requires immediate conventional medical attention (CMA). Scientific studies have not yet evaluated the effectiveness of CMA therapies in treating serum sickness. However, nutritional and herbal treatments may support conventional treatment by helping to reduce inflammation and stabilize the immune system. Many herbs have been mentioned in literature having anti inflammatory, antipyretic activity. It is widely believed that in order to prevent serum sickness during treatment of various pathological conditions, herbs can be effective in preventing the complications caused during serum sickness. Though herbs may not be the only drug, but
it can definitely support the treatment by antagonizing the side effects of the commercial mainstream therapy.

In cases of snake venom envenomation, patients die despite antiserum administration (Refer to case study I, page 52). It is therefore necessary to search for complementary substances to withstand the serum sickness and other drawbacks of the commercial antiserum. Herbs can be used in combination with the antiserum. Use of herbs can have the following advantages.

- No side effects
- Easily available
- Can be stored at room temperature
- Herbal extracts are easy to prepare
- Cost effective/cheap

It is therefore possible to suggest that the herbal antidotes would be useful in cases of emergency, when herbs might help the victim even before antiserum treatment is administered. Also, when administered with the antiserum simultaneously, the patient might receive greater protection against venom induced pathological symptoms as well as compensate for the drawbacks of the commercial antiserum. Earlier, a study on antiserum action potentiation had been conducted by 2-hydroxy 4-methoxy benzoic acid isolated and purified from methanolic root extract of *Hemidesmus indicus* which effectively potentiated viper venom induced lethal action (Alam *et al.*, 1998). It was observed that the degree of protection was higher when the herbal product was used in synergy with the antiserum to test venom induced lethal action in male albino mice.

In the present investigation, antiserum action potentiation study with the three plant extracts was undertaken. The crude extract of *Pluchea indica* root, *Hemidesmus indicus* root and *Strychnos nux vomica* seed were found to significantly potentiate antiserum action against both cobra and viper venom induced lethal action. Detailed antiserum action potentiation was conducted with the active constituents that are isolated and purified from the crude extracts.

Viper and cobra venom induced lethal action was antagonized by commercial antiserum upto $2LD_{50}$ of both the venoms. PINF1 incubated with
antiserum was injected (i.v) in male albino mice gave protection upto 6.6 LD_{50} of viper venom and 6.4 LD_{50} of cobra venom thus potentiating upto 330% and 320% respectively, with respect to lethal action of antiserum alone. Similarly, antiserum potentiation study was conducted with all the other active constituents. PINF2 potentiated antiserum action of viper venom induced action upto 520% and cobra venom induced action upto 355%. HINF1 and HINF2 isolated and purified from root extract of *Hemidesmus indicus* significantly potentiated antiserum action against viper venom induced lethality upto 205% and 190% and cobra venom induced lethality upto 415% and 240% respectively, with respect to lethal action of antiserum alone. SNVNF isolated from seed extract of *Strychnos nux vomica* also potentiated antiserum action against viper venom induced lethality upto 250% and cobra venom 190% induced lethal action, with respect to lethal action of antiserum alone.

The aim of this study was, to obtain greater degree of protection in snake envenomation. The present observation in antiserum action potentiation study is of immense significance from the therapeutic point of view. As mentioned already, antiserum is the only therapy available to the snakebite victim. Though a viable antidote, it does not give protection against high venom dose. Whereas, the antidotes isolated from plant sources gave greater protection against both viper and cobra venom induced action. When both are used together to test against venom induced lethal action in male albino mice, a much higher degree of protection was obtained. Since, a higher degree of protection against lethal action induced by snake venom is always desirable, it would be safe to suggest that when the plant product is used in synergy with commercial snake venom antiserum, it aims at a greater degree of protection and also the side effects caused by antiserum are neutralized, thus giving double benefit.

To summarize, in the present investigation certain selected Indian medicinal plants were found to possess antisnake venom activities. The methanol/ethanol extract of ten plant materials were found to possess antisnake venom activities against both viper and cobra venom induced lethal action in experimental animals. The plants are *Andrographis paniculata* Nees (aerial parts), *Aristolochia indica* Linn (root), *Crotalaria juncea* Linn (flower), *Croton*
tiglium Linn (seeds), Dolichandrone spathacea (L.f) k.Schum (seeds), Emblica officinalis Gaertn (root), Hemidesmus indicus R.Br (root), Pluchea indica Less (root), Strychnos nux vomica Linn (seed) and Vitex negundo Linn (root). Out of the above ten plants, Hemidesmus indicus R.Br (root), Pluchea indica Less (root), Strychnos nux vomica Linn (seed) were found to have the highest neutralizing capacity. These three plants materials were taken for isolation and purification of active constituents. Two active compounds were isolated and purified from the root extract of Pluchea indica. One of the two compounds have been identified as a mixture of β sitosterol and stigmasterol. The other compound was found to be a mixture but exact structure could not be identified. Both the two compounds were very potent against viper and cobra venom induced action in experimental animals. The methanolic root extract of Hemidesmus indicus was also taken for isolation and purification studies. Two active fractions were purified. One of them was identified as lupeol acetate and the other was a mixture and exact structure not elucidated. Both the compounds that were isolated were very potent against viper and cobra venom induced action in experimental animals. Study of the mechanism of venom inhibition suggested that the compounds inhibited the changes in serum transaminase and phosphatase levels, lipid peroxidation and superoxide dismutase levels caused by snake envenomation in experimental animals. One active fraction was isolated from ethanolic seed extract of Strychnos nux vomica which showed potent antitoxin venom activity against viper and cobra venom induced action. Detailed structure elucidation of the compound is pending. All the active constituents potentiated snake venom antiserum action against venom induced lethal action.

In conclusion, the present findings have validated some of the claims of antitoxin venom activity of selected Indian medicinal plants made by traditional literature and healers. Use of these plants to treat snakebite victims might work in cases where antiserum administration is ineffective or unavailable. Snakebite cases are very common in the rural areas and the victims die due to lack of proper treatment and high cost. Herbal antidotes if administered in proper dose
levels and frequency, might prove effective in saving a precious life. The present investigation was an effort to identify some of the Indian medicinal plants which are widely grown locally, having antisnake venom activity. The entire study was carried out in animal models and therefore requires further investigation. The use of the active compounds isolated from plants, for treatment cannot be recommended prior to clinical trials, which is not under the purview of the present investigation.

This study therefore proposes the use of medicinal plants to combat the major sociomedical problem of snakebite. Proper identification of the plant might allow the cultivation of the selected species in protected areas, so that indiscriminate destruction of the flora is avoided. In this way, the natural wealth can be utilized without affecting the ecological balance or making them extinct. Structure elucidation of the active constituents enables synthetic production of the compound on a large scale, thus providing cheap alternative to snake venom antiserum.

The study also suggests the use of these herbal antidotes in synergy with snake venom antiserum, to provide greater protection against venom induced lethal action. This was found to be very effective in animal models. Therefore it can be suggested that, the plant extracts may be useful in those areas where antiserum is not available, to prolong the time period till specific support is administered as well as potentiating the antiserum action to facilitate a higher degree of protection. The present investigation opens up a new dimension in venom research especially in its antagonism. Further investigation is necessary to establish more and more active plant constituents and detailed understanding of their mechanism of action.

The major findings of the present investigation:

- This study validates the claim of medicine men and traditional literature that there are Indian medicinal plants which can neutralize cobra and viper venom induced activities in experimental animals. Ten Indian medicinal plants have been identified to possess antisnake venom activity. Three plants with highest neutralizing capacity were selected for further studies.
Active constituents have been isolated and purified and structure of the compound elucidated from three plant materials. These three plants are *Plucaea indica* root, *Hemidesmus indicus* root and seeds of *Strychnos nux vomica*.

Two compounds, mixture of β-sitosterol and stigmasterol and a mixture of long chain compounds have been isolated and purified from the root extract of *Plucaea indica*. From the root extract of *Hemidesmus indicus*, two compounds were isolated and purified. One of them had been identified as Lupeol acetate and the other active compound is a mixture of triterpenoids. A small 3C compound has been isolated from the seed extract of *Strychnos nux vomica*.

The pure compounds isolated and purified from the three plants mentioned above were found to significantly neutralize both viper and cobra venom induced action in experimental animals.

The mechanism of venom inhibition was partly done by inhibiting venom induced free radical formation, change in serum transaminase and phosphatase levels.

The pure compounds were found to significantly potentiate antiserum action against venom induced lethal action.

**Future studies and proposals:**

Further studies are warranted before the herbal compounds are taken for clinical trials.

- Search for other plants with snake venom neutralizing capacity need to be continued.

- Further isolation and purification of antivenom compounds from the three plant extracts since it is believed that many more active constituents are present which can neutralize snake venom induced action.
• Isolation and purification method may be modified to obtain higher yield of pure compound.

• Newer experimental models both in vivo and in vitro need to be taken up to study the snake venom neutralization.

• Combination studies of snake venom neutralization with the isolated compounds need to be carried out in greater proportion to ensure greater protection against snakebite.

• Mechanism of venom inhibition need to be studied in detail.

• Derivatives of the isolated compounds may be studied for snake venom neutralization.

• Antiserum action potentiation by the pure compounds need to be taken up in other experimental models besides lethal action.