Research Article

Snake Bite, Venom, Anti-Venom Production and Anti-Venom Activity of Medicinal Plants: A Review

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ABSTRACT

Snakebite is a medically and socially significant issue in India, but it is one of the most neglected public health issues in poor rural communities. The estimated death incidence due to snake bites is 1,25,000 per year globally, which signifies that death rates associated with snake bites is a serious epidemiology. The current prospectus of treatment and reporting protocols need to be upgraded to higher standards. Anti-venoms have been widely used for more than a century for treating snakebites and other accidents with poisonous animals. Presently there are seven laboratories in India which produce anti-venom against four most venomous and medically important Indian snake species- Cobra *(Naja sp.)*, Krait *(Bungarus sp.)*, Russell's Viper *(Daboia russelii)* and Saw Scaled Viper *(Echis carinatus sp.)*, the 'big four'. Most venom for anti-venom production in India is sourced from Chennai. In this article, we review the production of venom and anti-venom in India and suggest areas of improvement, with the help of medicinal plants capable enough to be used as anti-venom. In light of these observations, it is felt that there is a need to prioritize the betterment of venom and anti-venom production protocols, public education, and snake bite treatment in India.

Keywords: Snake bite, Snake venom, Snake Anti-venom, Anti-venom production, Anti-venom activity of medicinal plants.

INTRODUCTION

Since ancient times, snakes have been worshipped, feared, or loathed. Unfortunately, snakes remain a painful reality in the daily life of millions of villagers. Indeed, although anti-venom is produced in sufficient quantities by several public and private manufacturers, most snake bite victims don't have access to quality care, both morbidity and mortality due to snake bites are high (Fig 1.). The neglected status of snake bite envenoming has recently been challenged[1] but as outlined below, apart from the production of anti-venom, snake bite envenoming in India shares all the characteristics of a neglected tropical disease.

This review aims at summarizing and discussing the epidemiology, clinical features, diagnosis, and treatment of snake bite envenoming in India.

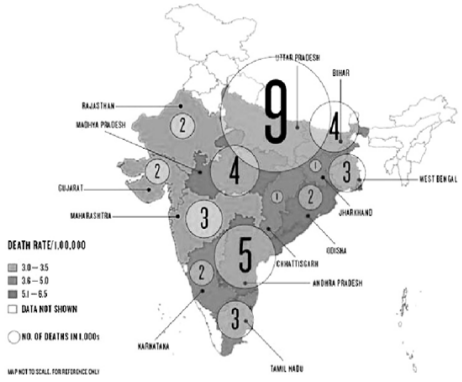

Figure 1: Estimated Mortality Due To Snake Bites In India

There are four common venomous snakes found in India. They are Common Cobra (*Naja naja*), Common Krait (*Bunguraus caeruleus*), Russell's Viper (*Daboia russelii*) and Saw Scaled Viper (*Echis carinatus*)[2] (Fig 2.). They are known as the "Big Four" of India. Apparently they are not the only species which are venomous, but are also found throughout India. They are widely distributed across the country. There are other venomous snakes like the King Cobra (*Ophiophagus hannah),* Hump Nosed Pit Viper (*Hypnale hypnale*), Banded Krait (*Bunguraus faciatus*) are also found in India.

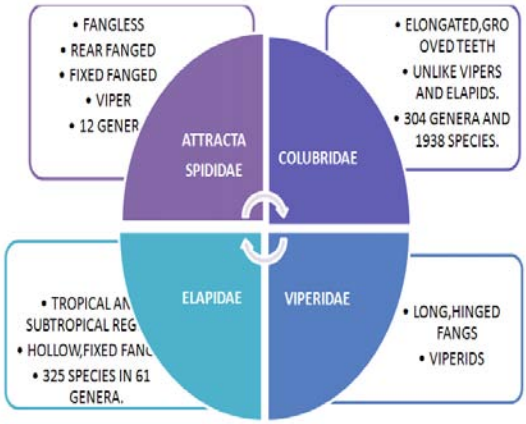

Figure 2: Characterization of Venom According To Snake Family

Death rate associated with snake bites is one of the major epidemiology in various regions of the world. About 35,000 to 50,000 people reportedly die of snake bite in India every year especially in rural areas[3] (Fig 3.).

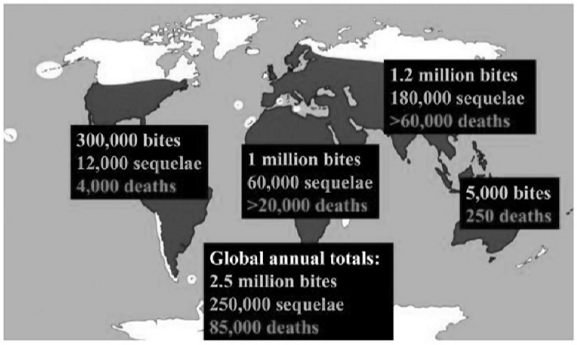

Figure 3: Statistics of Deaths by Snake Bites

Deaths due to snake bites may be reduced up to a certain extent by the use of proper first aid and medical support in the form of anti-venom. Despite increasing knowledge of snake venom's composition and mode of action, good understanding of clinical features of envenoming and sufficient production of anti-venom by Indian manufacturers, snake bite management remains unsatisfactory due to various downfall scenarios. Anti-venom's use may lead to significant side effects, anti-venom development is time consuming, expensive, and requires ideal storage conditions[4] and therefore search for venom inhibitors, either synthetic or natural, that could complement or substitute for the action of anti-venoms are of greater importance.

Snake Bite

A snake bite is basically an injury caused by a snake, often resulting in puncture wounds inflicted by the animal's fangs and sometimes resulting in envenomation. Although majority of snake species are non-venomous and typically kill their prey with constriction rather than venom, venomous snakes (15% out of 3000 known species)[5-7] are reported to be found on every continent except Antarctica[5].

Snake Bite Management

Health workers in rural districts are usually poorly trained to manage snake bite envenoming, which is a complex emergency. A recent survey conducted in India and Pakistan showed that many doctors were unable to recognize systemic signs of envenoming[8]. Another study in northwest India revealed that most snake bite victims presenting at primary health centres received inadequate doses of anti-venom and that out of 42 patients who required assisted ventilation, only one was incubated[9].

There are two important aspects of snake bite management.

Proper First Aid

Most experts agree that snake bite victims should be transported as quickly as possible to a medical centre where they can be clinically evaluated by qualified medical staff, and where anti-venoms are available. In fact, time of transport was shown to be a crucial determinant of snake bite mortality[10] and studies in southern India confirmed that delayed anti-venom administration was associated with an increased risk of complications[11,12]. The bite victim should be reassured, the bitten limb immobilized with a makeshift splint or sling, and the patient transported. Walking is contraindicated, because muscular contractions promote venom absorption.

Anti-venom Serum Therapy

Immunotherapy is the only specific treatment for snake bite envenoming. Anti-venoms are produced by fractionation of plasma obtained from immunized animals, usually horses[13]. They can be either monovalent or polyvalent, depending on the number of species (single or multiple, respectively) whose venoms are used for immunization.

Although monovalent anti-venom has often been considered more efficacious, the production of polyvalent anti-venom is preferred in many countries as snake species identification is generally not possible for the attending physician.

Anti-venoms have been available in South Asia for the past 60 years, and all existing products are manufactured by Indian companies.

Traditionally, the production has focused on four species believed to be responsible for most deaths: *N. naja*, *B. caeruleus*, *D. russelii*, and *E. carinatus*. However, a number of other species that contribute to morbidity and mortality in the region have not been considered, and envenoming by these species usually does not respond adequately to existing anti-venoms[14-16].

Snake Venom

Snake venom is essentially highly modified saliva that is made up of 90% proteins (by dry weight) and most of the proteins are enzymes[17]. Most of these enzymes are harmless to humans and are generally not dangerous when ingested.

There are about 20 toxic enzymes known to man and unique mixture of these zootoxins and proteins, form the lethal weapons of snakes. The venom contains phosphodiesterases (attacks the cardiac system), cholinesterase (loss of muscle control), hyaluronidase (increased tissue permeability), ATPases (disrupts energy fuel use) and various toxins (neurotoxins, cardiotoxins, blood clotting toxins, bleeding toxins) and other enzymes a major components, as well as small peptides, amino acids, carbohydrates, lipids, nucleosides, biological amines, metal ions and proteases[28]. It is stored in a large sac like structure, known as the alveoli behind the animal's eyes and it is ejected through a set of tubular fangs.

Fresh snake venom is neutral or weak acid, and it is alkaline when it is placed for a long time, and on exposure to air fresh venom produces foam and will be non-venomous and putrid when kept at room temperature for

24 hours[18] (toxicity disappear following UV irradiation and heat treatment; dealt with formaldehyde the toxicity also disappear but antigenic property is retained).

Classification of Snake Venom

Snake venom can be broadly categorized into many types, but the most considerable types are:

Hemotoxic Venoms

These attack the cardiovascular system, circulatory system and muscle tissues, thus directly leading to heart failures[19]. Normally, neither pain nor any other symptoms can be celebrated for almost 1 - 3 hours (sometimes even 8 hours). This makes it deadlier, as the victim is usually beyond medical help, by the time the cause is even ascertained. The effects can be seen as lethargy, headaches, nausea, vomiting, etc. The scariest observations of the outcome of snakebite of this kind are bruising or blood spots beneath the victim's skin. In extremely bad cases, blood is known to ooze out from all possible bodily openings. It is these venoms that usually cause excessive scarring, gangrene and permanent or temporary loss of motor skills. Worst cases can even result in the amputation of the affected limb[20].

Neurotoxic Venoms

They go after the central nervous system and brain. They often result in respiratory paralysis and heart failures. Their effect can range between mild seizures to death[21]. Cobras, mambas, sea snakes, kraits and coral snakes are known to possess it. The king cobras (*Ophiophagus hannah*) are the most infamous carriers of this venom. Neurotoxic venom is essentially nerve destroying. Hence, one can see speech and swallowing difficulties, drooling, difficulty in breathing, respiratory arrests, convulsions and sometimes even prolonged unconsciousness in the victims. The milder symptoms are dizziness, tunnel vision, blurred vision and increased sweating. It causes a very fast degeneration of the synaptic nerves and this is the reason for the blockage of nerve impulses sent to and from the brain to the muscles[21].

Cytotoxic Venoms

This is a milder form that generally causes only localized symptoms at the location of the bite. This is a cell destroying poison that destroys everything in its path - blood vessels, cells and tissues[22].

The symptoms of the invasion of this venom are generally seen around 10 - 15 minutes after the snake encounter.

The results are generally localized pain accompanied by severe swelling and bleeding. One can easily spot the formation of red blisters near the bite area. This venom causes blue/black spotting due to limited blood circulation. The body often revolts against the invasion of this venom by causing nausea and vomiting. If this is not treated within four hours, it generally needs an amputation[23].

Myotoxic Venoms

This venom is found in *Bothrops moojeni*, commonly known as the Brazilian lancehead snakes. It is known to cause muscular necrosis. Its symptoms are a thickened-tongue sensation, dry throat, thirst, muscular spasms and convulsions. It also causes the stiffness of the jaw, neck, trunk and limbs along with severe pain in movement[24]. The victims often start with drooping eyelids and then turn to more severe results like loss of breath and blackish brown urine discharge. Mitotic venom contains peptides that destroy the muscle fibre proteins and result in my necrosis (muscle destruction). In the very later stages (when treatment is delayed) of the spread of this venom, the muscle proteins enter the blood stream. The kidney overworks in trying to filter out the toxins eventually causing kidney failure which ultimately is the reason for the dark coloration of urine[24].

Venom Collection

In India, the Wildlife Protection Act provides in-situ protection for all snakes and as such, snakes cannot be collected for venom extraction or any other purposes without prior permission of the state wildlife authorities[25]. There is no scientific study that adequately quantifies snake abundance (through the export of up to 10 million snake skins per year in the 1960s gives some indication), which has resulted in a conservative stance by the wildlife authorities in some states and a general reluctance to permit capture of large numbers of snakes for venom extraction to produce Antivenins or Anti-venoms.

Snake Anti-Venom or Antivenin

Anti-venom first developed by Calmette (1895) aimed to neutralize venom toxins and was experimented against Indian Cobra (*Naja naja*). *Anti-venom* (or antivenin or antivenin) is a biological product used in the treatment of venomous bites or stings. It is a purified fraction of immunoglobulin or immunoglobulin fragments fractionated from the plasma of animals that have been immunized against a venom (venom from: snake, spider, scorpions etc)[26].

Anti-venom Types

Anti-venoms can be classified into monovalent (when they are effective against a given species' venom) or polyvalent (when they are effective against a range of species, or several different species at the same time) types.

Anti-Venom Production

Anti-venom production started in 1890's by Albert Calmette, who was living in present-day Vietnam when a flood forced monocle cobras into a village near Saigon, where they bit at least 40 people and killed four. Inspired by the then-new science of vaccinations, by 1896 Calmette had discovered the process of injecting horses with venom until they produced antibodies, taking the

serum out of their blood and injecting it into snake-bitten humans as anti-venom.

Basic Protocol for Antivenin Production

The first step in anti-venom production is milking venom from the desired snake, spider or insect. The venom is then diluted and injected into a horse, sheep or goat. The subject animal will undergo an immune response to the venom, producing antibodies against the venom's active molecules which can then be harvested from the animal's blood and used to treat envenomation[27]. Internationally, antivenins must confirm to the standards of pharmacopoeia and the World Health Organization (WHO)[28].

The basic protocol for anti-venom production includes following steps:

Milking the Venom

- The first step involves transferring of the captive or quarantine snake into a clean milking room.
- Next the snake is grabbed with the thumb and the index finger at the very back of its head, where the venom glands reside and the venom glands are pressed to ejaculate the venom through a plastic or rubber film into the vial (glass ware).

Cooling Down and Labeling

- After milking the venom, the venom is cooled to below -20^{o} C and freeze dried (lyophilized) for easier storage and transport. This will concentrate the venom and remove the water.
- Labeling of the venom from one particular species should be done because the anti-venom produced from particular venom is specific for it.
- It also prevents from being mixed with the anti-venom produced from the sub species of the same species.
- The label should contain the following information: Specificity of anti-venom, plasma unit number and date of collection.

Choosing an Animal for Immunization

Immunization is the process by which an individual's immune system becomes fortified against an agent (immunogen). In this context the selected animal is allowed to produce antibodies against the specific immunogen (snake venom).

Generally, horse (*Equus)* is the preferred animal:

- They thrive in many environment worldwide
- They have a large body mass
- They have long lives
- Comparatively big veins and friendlier
- Easy to handle

Other animals which can be used for the purpose of Immunization are Rabbit, Donkey, Goat and Sheep.

Immunizing

- A particular amount of venom along with distilled water, buffer solution (0.2 M Tris HCl) and adjuvant (a substance which enhances immune response e.g. Nanostructured silica, cobalt-60) is measured and injected into the horse.
- The amount of venom to be injected is divided into small volumes and injected separately into different organs where antibodies are produced (back of the neck - lymph nodes) to prevent ulcer or sore skin and maximize the area of immune reaction.
- The process of immunizing depends upon
  - ✓ Type of anti-venom
  - ✓ The snake used
  - ✓ The sort of antibodies desired

Purification

Blood is taken from the immunized animal and is centrifuged to separate plasma from the blood cells.

Basically, it is the plasma which contains effective antibodies against the venom.

The plasma is the filtered and remaining blood cells may be injected back into the animal.

The anti-venom thus produced is required to be separated from unwanted proteins. This is done though:

- Precipitation- The precipitate contains unwanted amino acids and proteins, which are then discarded off.
- Adjusting the Plasma's pH to 7.4- This step is required to maintain the neutral pH of the solution containing plasma.
- Adding salts (ammonium sulphate, hydroxylapatite etc) - Salts stabilizes the solution.

Breaking down of antibodies into small parts isolates its active ingredients consisting of the required anti-venom.

The anti-venom should be deemed safe and effective by the Food and Drug Administration.

Human Use

- The purified anti-venom is lyophilized and refrigerated in vials.
- In case of emergency the vials are filled with saline solution and injected intravenously (near the bitten region).
- A patient envenomed by snake bite requires 25-30 vials of anti-venom to be healed (1 vial = 6000 antivenin units).

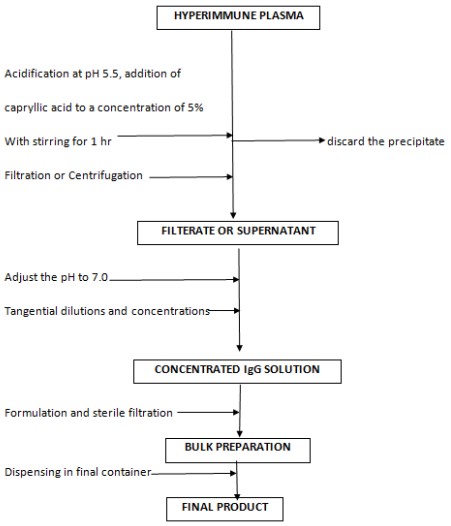

Figure 4: Flowchart: Fractionation of Plasma for Purification of IgG

Stability and Storage

- Stability is essential to determine the shelf-life of the product and intends to prove that anti-venom remains stable and effective.
- Quality control parameters determined at regular time intervals are venom neutralization potency, turbidity and content of aggregates.
- Anti-venom is stored at temperature within a range that assures the stability.
- For liquid preparations, requires storage temperature at between 2˚ and 8˚ Celsius.
- Interruptions in temperature may lead to deterioration.

Anti-Venom Producing Centres in India

- Bengal Chemicals and pharmaceuticals Ltd.- Kolkata
- Central Research Institute of Kasuli - Kasuli
- Haffkine Biopharmaceutical Co - Mumbai
- King Institute - Chennai
- Vins Bio-products Ltd.- Hyderabad
- Biological 'E' Ltd.- Hyderabad

Limitations of Anti-Venom

- Cause various side effects.
- Cannot undo damage already caused by venom, so anti-venom treatment should be started as soon as possible.
- Mostly administered intravenously but the route may not be uniformly effective.
- Production is time consuming and expensive.
- Liquid anti-venom may lose its activity due to protein precipitation, if not stored properly.
- Must be preserved always as freeze-dried sample.
- Anti-venom is unable to reach in remote areas.
- Inefficiency in reaching some countries due to storage difficulties.
- Resource intensive, pain staking.
- Complications in identifying the type of anti-venom to be used.
- Allergic reaction to certain individuals.
- Non-availability of anti-venom in hospitals.

Side Effects of Anti-Venom

Side effects of anti-venom therapy are anaphylactic reaction (difficulty in breathing and swallowing; hives; itching especially of feet or hands; reddening of skin, especially around the ears; swelling of eyes, face, or inside of nose; unusual tiredness or weakness), serum sickness (enlargement of the lymph glands; fever; generalized rash and itching; inflammation of joints), pyrogen reaction - probably due to the action of high concentrations of non-immunoglobulin proteins present in commercially available hyper immune venom[19].

Other Applications of Snake Venom

- Treatment of Breast Cancer
- Treatment of Blood Clots
- Used as Morphine and Anaesthesia
- Treatment of Alzheimer's Disease

Snake venoms are used to control heart diseases, high blood pressure, cancer (contortrostain produced by *Agkistrodon contortrix* - is cytostatic in nature and found to lower the growth of breast cancer in mice), tumor, polio, neurological disorders (enzymes from cobra venom were found to cure Parkinson's and Alzheimer's diseases), excessive bleeding (a blood clotting protein in Taipan venom stop bleeding during surgery or after major trauma), blood clotting (ancrod - obtained from Malyan pit viper, used to develop angiotensin converting enzyme inhibitors to treat stroke victims), severe allergies amongst others[29]. Other interesting areas of snake venom include the treatment of viruses (as venom contains phospholipidases which break down cell membrane), aging and some are even used in commercial wrinkle cream[30].

Case Study

According to Leslie Boyer a physician and head of the Viper Institute at the University of Arizona, the process is much improved but the steps largely remains the same. Efforts are continuously being made to produce anti-venom for the world's deadliest snake bites. There's one other, quirkier way to make anti-venom–one that

physicians don't exactly recommend. For decades Bill Haste, milked about 100 snakes a day with his bare hands, and in 1948 began injecting himself with increasing doses of diluted cobra venom in order to develop his own immune resistance. At the time of his death (not caused by snakebite), he'd survived 172 bites from many of the world's deadliest snakes, including a blue krait, a king cobra and a Pakistani pit viper. He flew around the world to donate transfusions of his antibody-rich blood to treat 21 snakebite victims. Venezuela made him an honorary citizen after he traveled into the jungle to donate blood to a young snake-bitten boy. According to his wife Nancy, all 21 patients survived[31].

Anti-Venom Activity of Medicinal Plants

The use of plants against the effects of snakes bite (Table 1.) has been long recognized; more scientific attention has been given since last 20 years[32]. Extracts from plants have been used among traditional healers, especially in tropical areas where there are plentiful sources, as therapy for snakebite for a long time. Several medicinal plants, which appear in old drug recipes or which have been passed on by oral tradition, are believed to be snakebite antidotes. In modern science, there have been many attempts to study these plants to clarify their effectiveness. India has a rich tradition of the usage of medicinal plants.

Many Indian medicinal plants are recommended for the treatment of snakebite and their anti-venomic properties were confirmed by many scientists. The methanolic root extracts of *Vitex negundo* and *Emblica officinalis* were tested for anti-venom activity. Both plant extracts were able to significantly neutralize the *Vipera russellii* and *Naja kaouthia* venom induced lethal activity, both *in vitro* and *in vivo* studies[33]. *V. russellii* venom-induced haemorrhage, coagulant, defibrinogenating and inflammatory activity was significantly neutralized by both plant extracts[34]. *Hemidesmus indicus* root extracts effectively neutralized Viper venom induced lethal, haemorrhagic, coagulant anticoagulant and inflammatory activity[35]. The butanolic extract of *Eclipta prostrata* plant partially inhibited the hemorrhagic activity but displayed very low antiphospholipase A2 activity and did not inhibit proteolytic activity of Malayan pit viper venom[32, 36]. Lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* could significantly neutralize lethality, haemorrhage, defibrinogenation, edema, PLA2 activity induced by *Daboia russellii* venom. It also neutralized *Naja kaouthia* venom induced lethality, cardio-toxicity, neuro-toxicity and respiratory changes in experimental animals[37]. Beta-sitosterol and stigmasterol isolated from the root extract of *Pluchea indica* may play an important role, along with antiserum, in neutralizing snake venom-induced actions.

Several plant constituents like flavonoids, quinonoid, xanthene, polyphenols and terpenoids possessed protein binding and enzyme inhibiting properties and also inhibit snake venom phospholipase A2 (PLA2) activities of both Viper and Cobra venom[27]. Triterpenoids present in *V. negundo* and *E.officinalis* may involve in venom inactivation processes. The pentacyclic triterpenes (free or as glycosides) are found widely in several plants possessing snake antivenomic properties (*Aegle marmelos*, *Centipeda minima*, *Aloe vera*, *Phyllanthus niruri*, *Alstonia scholaris*, *Phyllanthus emblica*, *Elephentopus scaber* etc.) and provide nearly 20% protection against snake venom[38]. *Eclipta prostrata* is a tropical and subtropical plant used as anti-venom against snakebite in China and in Brazil[39]. It was reported that the methanolic extract from *Cordia verbenacea* significantly inhibited paw edema induced by *Bothrops jararacussu* snake venom[40]. An active compound from the *Strychnus nux vomica* seed extract, inhibited viper venom induced lipid peroxidation in experimental animals. Jantropha Caracas, Acacia leucophloea, Albizia procera, Madhuca indica and Ocimum sanctum plants are applied as an anti snake in Orchha wildlife sanctuary region of Tikamgarh District, Madhya Pradesh, India[14]. Total six medicinal plants used in the treatment of snake bite reported in Taindol village, district Jhansi, region of Bundelkhand, Uttar Pradesh, India[41].

It may be concluded that evidence are now available to establish the scientific background of the traditional use of plants against snakebite. The anti snake venom plants contain more than one compound (secondary metabolites) that are responsible for venom neutralization. Thus medicinal plants with anti-venom activity could be considered as an effective alternative to mammalian antibody production for the treatment of snakebite envenomation.

Table 1: Some of the Most Needed Anti-Venoms

	Common Name	Species Name	Anti-venom(s)	Threat
America	Lanceheads	*Bothrops* species	Antibotropico Polyvalent	Responsible for 15 deaths per 100,000 people in Brazil
Africa	West African carpet viper	*Echis ocellatus*	FAV-Afrique, SAIMR Echis anti-venom	Most common snake danger in central and west sub Saharan Africa, up to 12.3% fatality rate (in Nigeria)
Asia	Malayan pit viper	*Calloselasma rhodostoma*	Haemato-Polyvalent snake anti-venom, Malayan pit viper, Polyvalent anti snake venom	Responsible for half of all hospitalizations in Malaysia, where mortality rate reaches 18 per 100,000 people
Oceania	Australian brown snakes	*Pseudonaja* species	CSL brown snake anti-venom, CSL polyvalent anti-venom	Responsible for half of approximately 200 snakebite deaths in Oceania each year

CONCLUSION

In prospect of the number of deaths induced by snake bite, especially where anti venom is not readily accessible, the development of cheap remedies suitable for emergency handling is significant. The veracity of the herbal assertions holds a good promise for the development of novel snake anti-venom drug in future. The combinations of herbal compounds with anti-venom serum may also be a good prospective as well as effective in neutralizing snake venom. Most importantly medicinal plants possessing anti-venom activity should be properly identified and cultivated, and knowledge must be disseminated properly so that at least first aid treatments can be provided to reduce mortality of snake bite.

Till date proper herbal formulations and its efficacy in relation to remedial measure against snake bites are yet not known properly, and research should be triggered in this direction.

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