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A POTENTIAL APPROACH ON SNAKE VENOM POISONING AND ANTIVENOM

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ABSTRACT

In the world, out of 3000 species of snakes, only 450 are venomous. Snake venom is a type of poison that is typically yellow fluid and is produced in the back of the snake's head in the salivary gland, the parts of the head where saliva is made. It is composed of proteins enzymes and other molecular substances. These toxic substances work to destroy cells and disrupt nerve impulses or both. Snakes use their venom for hunting prey or for defending against predators. Once the snake bites, muscles in its head squeeze the venom gland. This pushes the liquid through its hall of fangs muscles that act as hypodermic needles and inserts the venom into the flesh of its spray. Once the venom is injected, some toxins target the nervous system which is called neurotoxins, Hemotoxin, Myotoxin. So it is essential to have knowledge about different species of snakes so that doctors could provide the right kind of anti-venom which is the cure for a snake bite the anti-venom is actually made out of the venom itself to develop an anti-venom a host of the animal usually a horse is injected with larger amounts of venom eventually the horse's immune system causes blood plasma cells to produce antibodies these antibodies are then extracted from the host animal's body processed and purified so that they can be given to snake bite victims the king cobra is the largest snake in the world with the ability to inject venom also the inland taipan is considered the most venomous snake in the world.

KEYWORDS: Snake venom, Snakebite, Enzymes, Antivenom, Toxins.

INTRODUCTION

Across many tropical and subtropical nations, snake bite is a neglected public health concern. An estimated 5.4 million snake bites result in 1.8 to 2.7 million envenoming cases per year (poisoning from snake bites). Between 81 410 and 137 880 people die every year, and there are about three times as many amputations and other types of lasting disabilities.

The majority of these occur in Latin America, Asia, and Africa. Each year, up to 2 million individuals in Asia and between 435 000 and 580 000 people in Africa are bitten by snakes and require medical attention. Envenoming affects women, kids, and farmers in underdeveloped rural areas of low- and middle-income nations. The largest impact occurs in nations where health systems are weakest and medical resources scant.

Venomous snake bites can cause extensive local tissue damage, irreversible renal failure, severe paralysis that may prohibit breathing, bleeding problems that may result in deadly haemorrhage, and severe bleeding that may result in limb amputation or lifelong handicap. Due to their lower body masses than adults, children may feel the consequences more immediately and with more severity.

Unlike numerous other significant medical diseases, this one has a very good therapy. Providing reliable and safe antivenoms that are more broadly obtainable and readily available, most deaths and significant repercussions from snake bites may be completely avoided. The best way to stop or reverse most of the poisonous consequences of snake bites is by using high-quality snake antivenoms. They are a feature of any basic healthcare plan where snake bites occur and are listed on the WHO List of Essential Medicines.

Snake venom contains zootoxins, which aid in immobilizing and digesting prey. It is an extremely poisonous saliva. Additionally, this offers threat protection. When a snake bites, its distinctive fangs inject venom; nevertheless, certain species may also spew venom.

Zootoxin-producing glands are modified parotid salivary glands that may be found in other vertebrates. They are typically placed on either side of the head, below and behind the eyes, and are encased in a muscular sheath. Before being transported by a duct to the base of channelled or tubular fangs, where it is released, the venom is first stored in huge glands called alveoli. The venom contains more than 20 compounds, mostly proteins and polypeptides. The complex mixture of proteins, enzymes, and various other substances has toxic and lethal properties. Venom serves to immobilize prey. Enzymes in venom play an important role in the digestion of prey, and various other substances are responsible for important but non-lethal biological effects. Some of the proteins in snake venom have specific effects on various biological functions, including blood coagulation, blood pressure regulation, and nerve or muscle impulses transmission. These venoms have been studied and developed for use as pharmacological or diagnostic tools, and even drugs.

There are an estimated 600 poisonous snake species in the globe out of the 3,500 kinds of snakes. This is a list of the snakes that can seriously harm people's health by biting them or causing severe bodily harm.

The kind of snakes that frequently result in severe snakebites vary by geographic location. The most hazardous animals in Africa include carpet vipers, puff adders, and black mambas. The most dangerous species are carpet vipers and elapids in the Middle East, while Bothrops and Crotalus (rattlesnakes) are the most dangerous in Central and South America. Indian cobras, common kraits, Russell's vipers, and carpet vipers have historically been thought to be the most hazardous snake species in South Asia; nevertheless, other snakes can also be quite dangerous in this region of the world.

1. MOST DANGEROUS SPECIES OF SNAKES

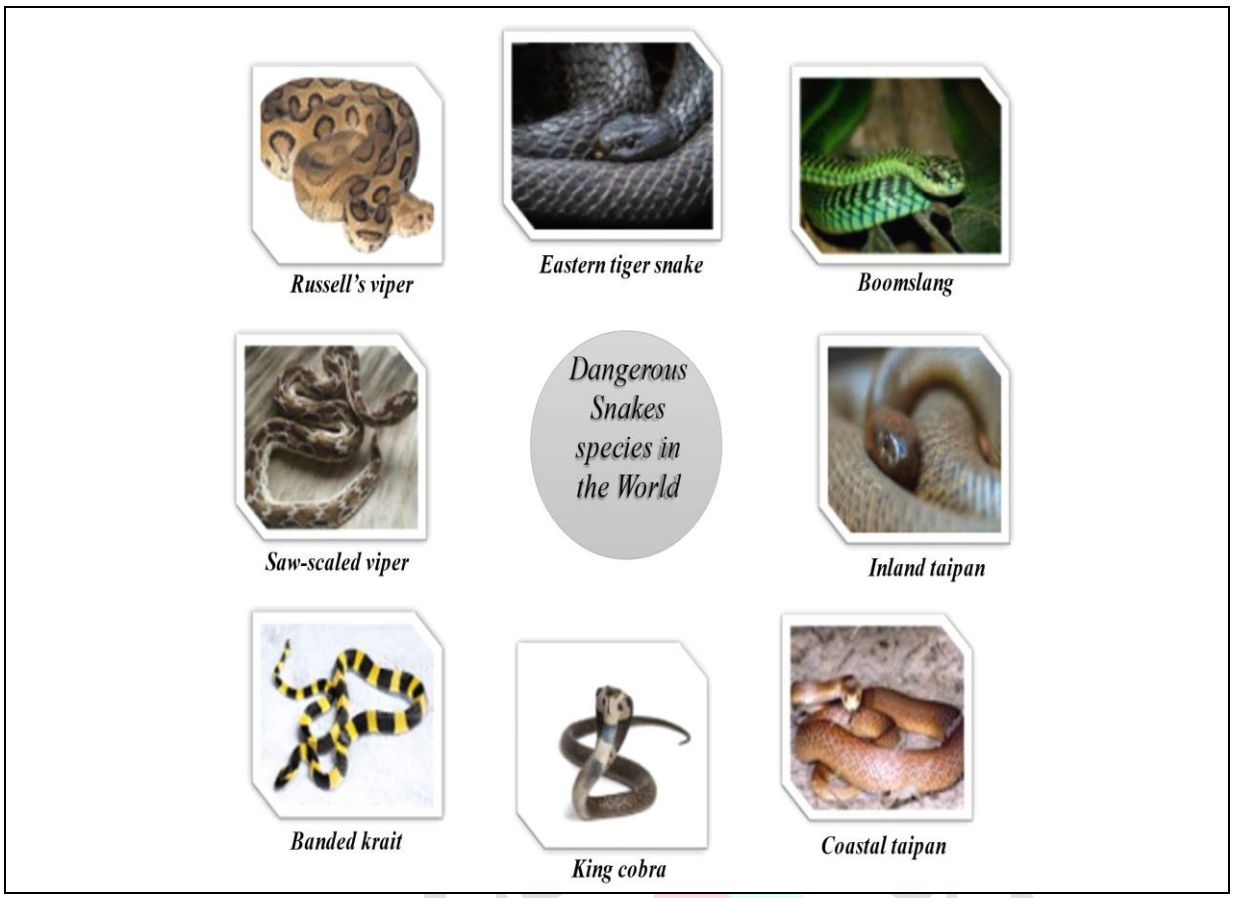


Figure No.1 : Dangerous Snake Species in World

Name of Snake	Biological Source	Geographical Existence	Active constituents of venom	Special features
1. Inland Taipan	Oxyuranus microlepidotus Family: Elapidae	Queensland and South Australia's floodplains.	β-bungarotoxin high MW. α-bungarotoxin low MW	Most toxic of all snake venoms and strongly neurotoxic.
2. Coastal taipan	Oxyuranus scutellatus Family: Elapidae	Wet forests of temperate and tropical coastal regions of Australia.	Taicatoxin, paratoxin. Procoagulants, Taicatoxin.	Longest snake species in Australia.
3. King Cobra	Ophiophagus Hannah Family: Elapidae	Southeast and Southern Asia. Northern India, east to southern China,	Cardiotoxin (cytotoxin) – β-structured protein.	Longest venomous snake. The

			south throughout the Malay Peninsula, and east to western Indonesia and the Philippines.		life span of 20 years.
4.	Banded krait	Bungarus fasciatus Family: Elapidae	Indian Subcontinent, in Southeast Asia, and in southern China.	Neurotoxins like Acetylcholinestearse, Phospholipase B, and Glycerophosphatase, α and β bungarotoxin.	Paralyze muscles and prevent the diaphragm from moving.
5.	Saw-scaled viper	Echis carinatus Family: Viperidae	Dry regions of Africa, the Middle East, India, Sri Lanka, and Pakistan.	Echicetin - heterodimeric protein. Hemotoxin, cytotoxin, a neurotoxin.	"sizzling" warning sound.
6.	Russell's viper	Daboia russelii Family: Viperidae	India, Pakistan, Bangladesh, and Sri Lanka, while Eastern, Southeast Asia and Southern China, including Taiwan.	'Russell's viper venom factor V activator' and a metalloproteinase 'Russell's viper venom coagulation factor X-activating enzyme (RVV-X)'.	Flattened, triangular head, blunt snout, two long fangs, large nostril.
7.	Eastern tiger snake	Notechis scutatus Family: Elapidae	Southeast Australia.	Neurotoxins, myotoxins, and procoagulants.	Before striking, the tiger snake flattens its head and neck, in cobra fashion.

8. Boomslang	Dispholidus typus Family: Colubridae	Africa but lives primarily in Swaziland, Botswana, Namibia, Mozambique, and Zimbabwe.	Hemotoxin. Hemotoxins.	Most venomous of the so-called rear-fanged snakes.

Table No. 1: Dangerous species of snakes

2. MECHANISM OF SNAKE POISONING :

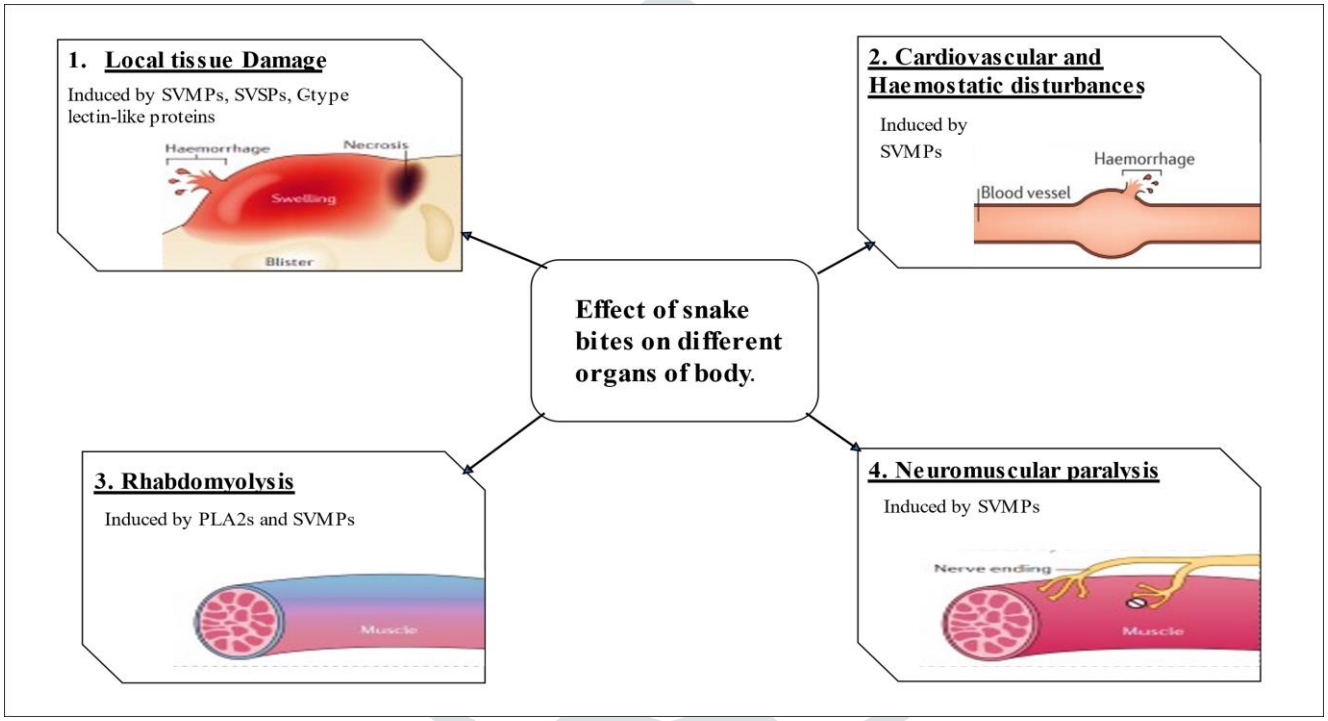


Figure 2: Mechanism of snake poisoning

The potential consequences of snake bites are discussed below –

- **Local tissue damage** –

Local tissue injury is caused by the majority of viperid and certain elapid venoms. Myotoxic phospholipase A2 (PLA2s), which are found in these venoms and bind to and compromise the integrity of the plasma membrane of muscle fibres, is the primary cause of myonecrosis. For certain PLA2s, disruption of plasma membranes results from the hydrolysis of membrane phospholipids, whereas hydrophobic interactions harm the sarcolemma in the case of PLA2 homologs that lack catalytic activity. Following membrane disruption, calcium influx into the cytosol results in myofilament hypercontraction, mitochondrial malfunction, and other degenerative processes that result in irreparable muscle cell destruction.

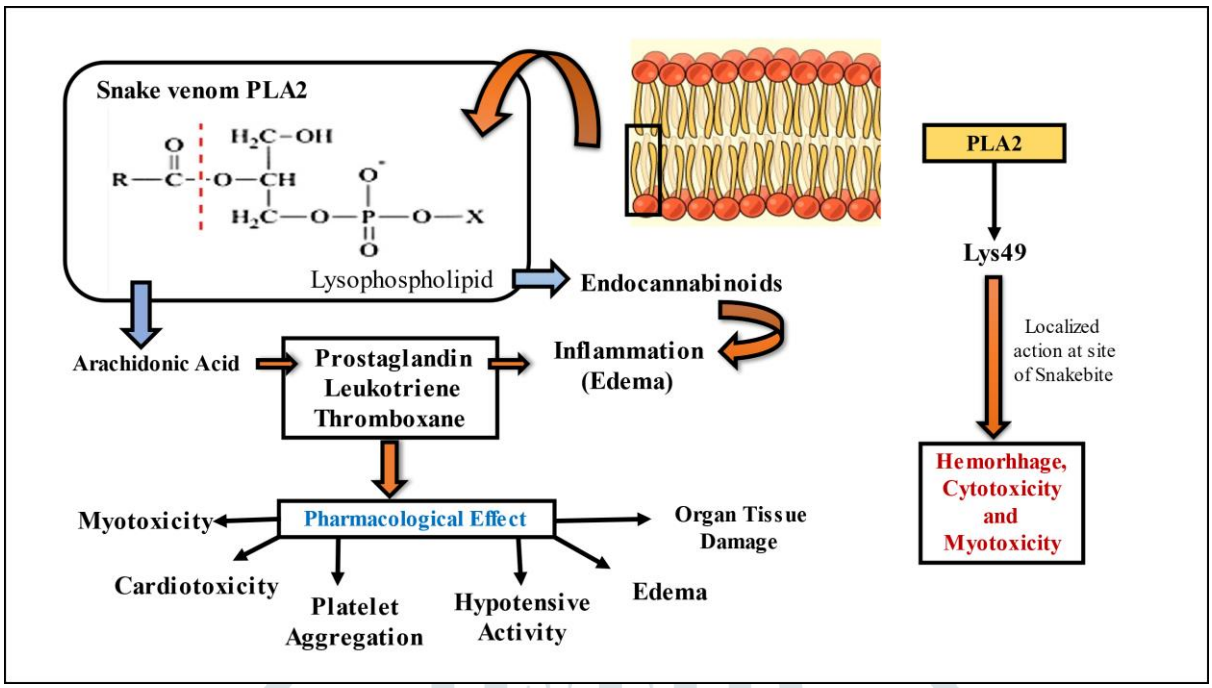


Fig 3: Action Of PLA2 (Phospholipase 2) on local tissue

- **Cardiovascular and haemostasis imbalance**:

Systemic haemorrhage can ensue after being envenomated by Australian elapids, several species of non-front-fanged colubrid, and viperids. The primary toxins of viperid venoms that cause systemic haemorrhage are SVMPs, particularly those of the class PIII. These toxins can target the microvasculature because of their multi-domain structure, which contains exosites (molecular sites apart from the active catalytic site that serves as secondary binding locations). Different organs may bleed, which can have various pathological effects. For instance, cerebral haemorrhage, which can result in ischemia, stroke, and neurological consequences, has been reported in envenoming the mechanism of action of systemically active haemorrhagic SVMPs is probably like that reported for local haemorrhage, that is, cleavage of important substrates at the basement membrane of capillaries and at cell-cell junctions, causing the mechanical weakening of the micro vessel wall and extravasation. Haemostasis is impacted by snake venom in several ways. These coagulation-promoting enzymes are either SVMPs or snake venom serine proteinases that act in the coagulation cascade, such as thrombin-like enzymes or activators of coagulation factor V, factor X, or prothrombin. Many viperid venoms, as well as some elapid and non-front fanged colubrid venoms, contain these enzymes. Fibrinogen and fibrin are hydrolysed by certain venom enzymes. Additionally, SVMPs produce tissue factor and have a variety of effects on endothelial function. Although these procoagulant substances can result in intravascular coagulation, they often produce consumption coagulopathy, which impairs coagulability and defibrinogenation and affects blood clotting assays. Systemic bleeding may be caused by this illness, especially when venoms include haemorrhagic toxins that damage blood vessel integrity. Systemic bleeding is frequently brought on by some Australian elapid venoms that lack haemorrhagic SVMPs but produce coagulopathy due to serine proteinase prothrombin

activators. The venom of several snakes affects platelets. The reduction in platelet counts is a result of C-type lectin-like proteins and microvascular injury caused by SVMP. Disintegrants, C-type lectin-like proteins, snake venom serine proteinases, and certain SVMPs also prevent platelet receptors from being accessed or interfere with von Willebrand factor, which reduces platelet aggregation. In envenoming by haemorrhagic venoms, thrombocytopenia has been linked to an increased risk of systemic haemorrhage. In contrast, although not being directly procoagulant, the venoms of two indigenous Caribbean viperid species cause severe thrombosis, which causes infarcts in the heart, lungs, and brain. Thrombosis is likely reliant on systemic endothelial dysfunction brought on by SVMP. Some viperid bites result in acute pituitary insufficiency due to thrombi development and localised haemorrhage in the anterior pituitary glands. One of the main contributors to the haemodynamic abnormalities that patients envenomed by viperids encounter, which can lead to cardiovascular shock, is venom-induced systemic bleeding. Hypovolaemia in these envenoming is a side effect of increased vascular permeability, which includes systemic plasma leakage.

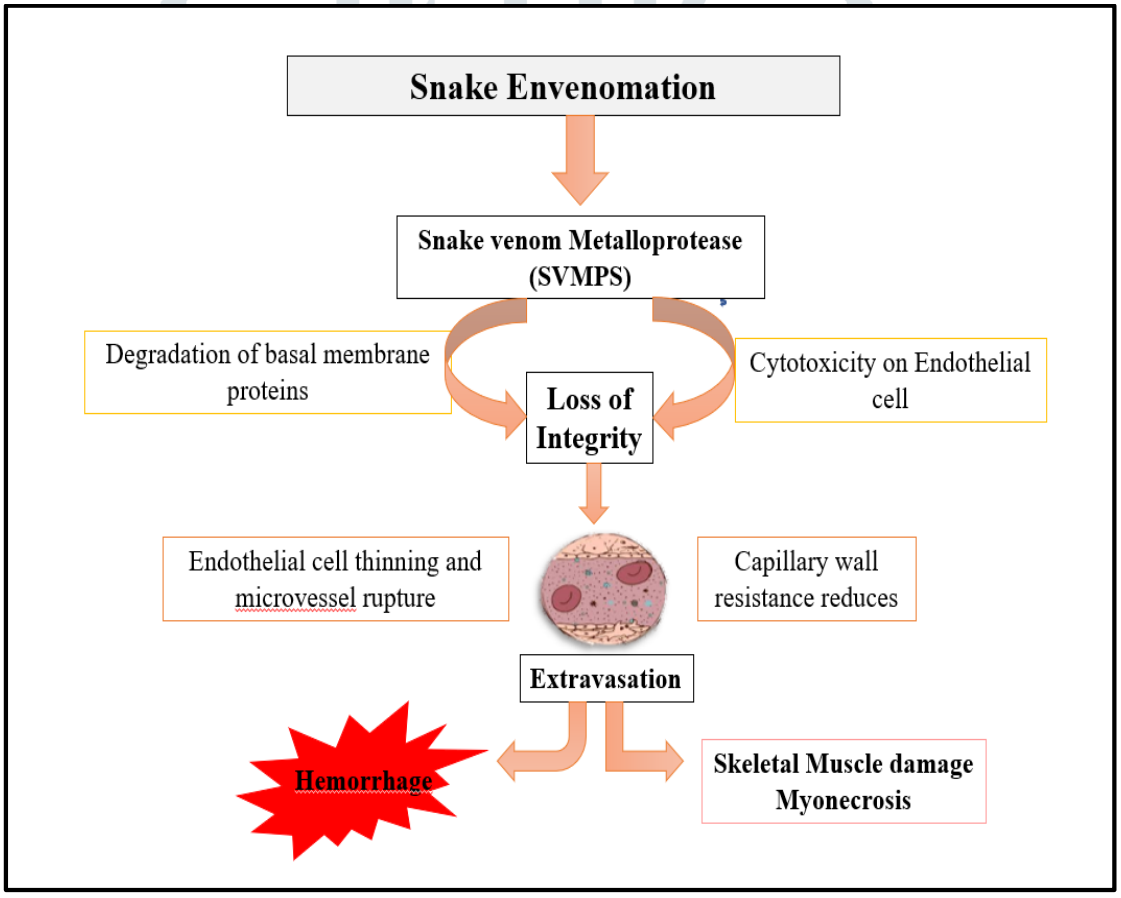


Fig 4: Action of Snake venom metalloprotease (SVMPs)

- **Rhabdomyolysis –**

Rhabdomyolysis is linked to envenoming by sea snakes, several Australian terrestrial elapids, and some viperid species. As a result of these toxins' binding to receptors on muscle fibres, myotoxic PLA2s exert their systemic myotoxic effects. According to the description of locally active mycotoxins, myotoxins

alter the integrity of the plasma membrane of muscle cells, leading to calcium influx and cellular aging. As a result, a lot of muscle cytosolic proteins are released, including myoglobin and creatine kinase. Myoglobin build up in the renal tubules may be a factor in acute kidney damage.

- **Neuromuscular Paralysis –**

Most elapid snake species as well as some viperid snake species venoms contain neurotoxins that cause a descending flaccid neuromuscular paralysis. This paralysis can be life-threatening and include the blockage of the bulbar (mouth and throat muscles responsible for speech and swallowing) and respiratory muscles. Neurotoxins and neurotoxins are the two primary categories of neurotoxins identified in snake venom. Neurotoxins operate postsynaptically at neuromuscular junctions and are members of the three-finger toxin family. They impede the binding of acetylcholine and cause flaccid paralysis by binding with a high affinity to the cholinergic receptor at the motor end plate in muscle fibers. On the other hand, neurotoxins often operate at the presynaptic nerve terminal of neuromuscular junctions and are PLA2s. For instance, a voltage-gated potassium channel is a receptor for bungarotoxin, which is found in the krait Bangaru multicinctus (family Elapidae). Neurotoxicity is brought on by the enzymatic degradation of phospholipids at the nerve terminal plasma membrane that neurotoxic PLA2s produce upon binding to their targets. Indeed, the production of lysophospholipids and fatty acids in the membrane results in biophysical modifications that allow synaptic vesicles to fuse to the membrane and the ready-to-release pool of vesicles to exocytosis. Additionally, there is an increase in membrane permeability to ions, which leads to a depolarization and calcium influx that exocytosis the reserve vesicle pool. Presynaptic vesicles are thus depleted, and intracellular degenerative processes, such as mitochondrial changes, result, leading to the loss of nerve terminals. These incidents explain the patients' severe and protracted paralysis. Some neurotoxic PLA2s can operate intracellularly after passing through the damaged plasma membrane or endocytosis to reach the cytosol. PLA2s promote further mitochondrial degenerative processes inside the nerve terminal. Dendrotoxins and fasciculations, which can be found in the venom of African mambas, are additional neurotoxins (Dendroaspis spp.; family Elapidae). The presynaptic nerve terminal's voltage-gated potassium channels are blocked by dendrotoxins. The three-finger toxin family member fasciculations are acetylcholinesterase inhibitors. These neurotoxins work together to produce excitatory effects and fasciculations (involuntary contractions of small groups of muscle fibres). Certain cysteine-rich secretory proteins in venoms cause smooth muscle paralysis.

3. DIAGNOSIS WITH LABORATORY AND OTHER INVESTIGATION –

These incidents explain the patients' severe and protracted paralysis. Some neurotoxic PLA2s can operate intracellularly after passing through the damaged plasma membrane or endocytosis to reach the cytosol. PLA2s promote further mitochondrial degenerative processes inside the nerve terminal. Dendrotoxins and fasciculations, which can be found in the venom of African mambas, are additional neurotoxins (Dendroaspis spp.; family Elapidae). The presynaptic nerve terminal's voltage-gated potassium channels are blocked by dendrotoxins. The three-finger toxin family member fasciculations are acetylcholinesterase inhibitors. These neurotoxins work

together to produce excitatory effects and fasciculations (involuntary contractions of small groups of muscle fibres). Certain cysteine-rich secretory proteins in venoms cause smooth muscle paralysis. Venom antibody detection and titration are typically carried out using indirect ELISA. This might help determine the potency and para-specific efficacy of antivenoms, assess the prevalence of snakebites and research the immune system's reaction to venom immunogens. Snake venom ELISA techniques now in use have little specificity and can often not effectively distinguish between venoms from related snakes. Assays for detecting venom antibodies are less successful than those for venom; cross-reactivity and nonspecific results are unacceptable.

4. COMMONLY ADOPTED APPROACHES FOR DIAGNOSIS OF SNAKEBITE ENVENOMING

A basic diagnosis of snakebite envenoming requires a thorough patient history, targeted examination, and appropriate laboratory investigations. Taking a detailed history includes asking about the circumstances of the bite (e.g. geography, time of the incident, activity, and number of bites), details of the snake (if seen, brought, or photographed), clinical manifestations of envenoming (including time of onset), first aid applied, and past medical history (e.g. allergies, prior snakebites, relevant medications, and pre-existing medical conditions). Laboratory investigations almost always include an evaluation of the blood clotting profile to screen for venom-induced coagulopathies. In its simplest form, a blood clotting test can be carried out in the form of a 20-minute whole blood clotting test (20WBCT). If more sophisticated equipment is available, it is common to run repeated tests of the International Normalized Ratio (INR) of blood clotting, activated partial thromboplastin time (aPTT), D-dimer, and/or fibrinogen degradation products (FDP), supplemented by hemograms and potentially also by electrocardiograms. Acute falls in hemoglobin and hematocrit values may indicate internal bleeding, and a drop in fibrinogen levels might be indicative of coagulopathy (7, 110, 111). Blood samples are usually also screened for creatine kinase (CK) levels, electrolytes, urea, and nitrogen/creatinine, which together with urinalysis (haematuria, proteinuria, urea levels, and urine output) can be used to assess venom-induced rhabdomyolysis and associated complications, such as myoglobinuric renal failure or polyuria, oliguria, or anuria.

Auxiliary tests	
Type	**Subtype**
Hemogram	Platelet count Blood count Examination of blood film for evidence of intravascular hemolysis
Clotting Profile	Fibrinogen level Prothrombin time Activated partial thromboplastin time
Serum Biochemistry	Electrolytes

	Bilirubin Liver function test Creatine kinase
Urinalysis	Haematuria Myoglobinuria
Renal function	Serum creatinine Urea Glomerular filtration rate Urine output
Electrocardiogram	ECG

Table No.2: Diagnostic test for detection of Snakevenom

5. DETECTION OF VENOM

Detection and quantification of venom antigens in body fluids of individuals with snakebite envenoming, using enzyme immunoassays provide retrospective confirmation of species diagnosis, predict prognosis, and is one measure of the effectiveness of antivenom treatment. High concentrations of venom antigens (that is, from wound swabs or wound aspirates) can be detected within 15–30 minutes, but commercial venom detection kits are available only in Australia (produced by Seqirus). Venom detection kits are highly sensitive but insufficiently specific to distinguish between venoms of closely related species. Detection of venom in a wound swab does not prove that the patient has been envenomed and is not, on its own, an indication for antivenom treatment. For retrospective species diagnosis, including forensic cases, tissue around the fang punctures, wound, and blister aspirate, and serum and urine samples should be stored for enzyme immunoassays. For determining the identity of the biting species, highly specific methods are being developed, such as the detection of venom gland mRNA by reverse-transcription PCR or snake-derived DNA in bite-wound swabs.

6. ROLE OF SNAKE VENOM DETECTION KIT (VDK)

- A VDK is rarely indicated as:
- There are only two types of antivenom required for Victorian snakes (tiger and brown) and both can be given to treat envenomation without identifying the snake.
- The diagnosis of envenomation is based on the history, examination, and laboratory test findings. A VDK is NOT used to diagnose envenomation
- A VDK may be indicated if the snakebite is from a non-Victorian snake
- Attempted identification of snakes by witnesses should never be relied upon as snakes of different species may have the same colouring or banding

- VDKs can have significant rates of snake misidentification with both false positives and false negatives and should therefore only be performed by an experienced laboratory technician
- The results should not override clinical and geographical data. Discuss use and results with a clinical toxicologist (e.g. Poisons Centre)
- If used, a VDK should be used on a bite site swab, and a single operator should be dedicated to performing the VDK interpretation and should do so free from other clinical responsibility and interruption. This takes 20-30 minutes, and as such should be omitted in the unwell or arrested child. A brief lapse in concentration when watching for color change in the VDK can result in a false reading
- If there is no apparent bite, a VDK may be done on urine, but never blood.

7. TREATMENT :

a. Location of care

Uncomplicated snakebites can be managed at a regional centre as long as the following resources are available:

- A doctor who is willing and able to care for the child 24 hours a day,
- Immediate access to critical care facilities,
- Immediate access to the required antivenom, and
- Access to a 24-hour pathology laboratory that can perform the required blood tests.

b. First aid

Apply a broad pressure immobilization bandage,

- Preferably elastic rather than a crepe, as firm as you would for a sprained ankle;
- The aim is to prevent venom's lymphatic spread, not stop the blood supply.
- Start at the bite site and bandage the entire limb.
- Immobilize the joints on either side of the bite site (use a splint),

  Immobilize the entire child as well (lay the child down). DO NOT remove the bandage until in a centre with full treatment facilities, as discussed above.
- If envenomed, do not remove until antivenom has been given.
- Once the antivenom has been given, remove the pressure immobilization bandage.
- Do not wash or clean the bite site in any way (in case the use of a Venom Detection Kit is required)

c. Pressure Immobilization

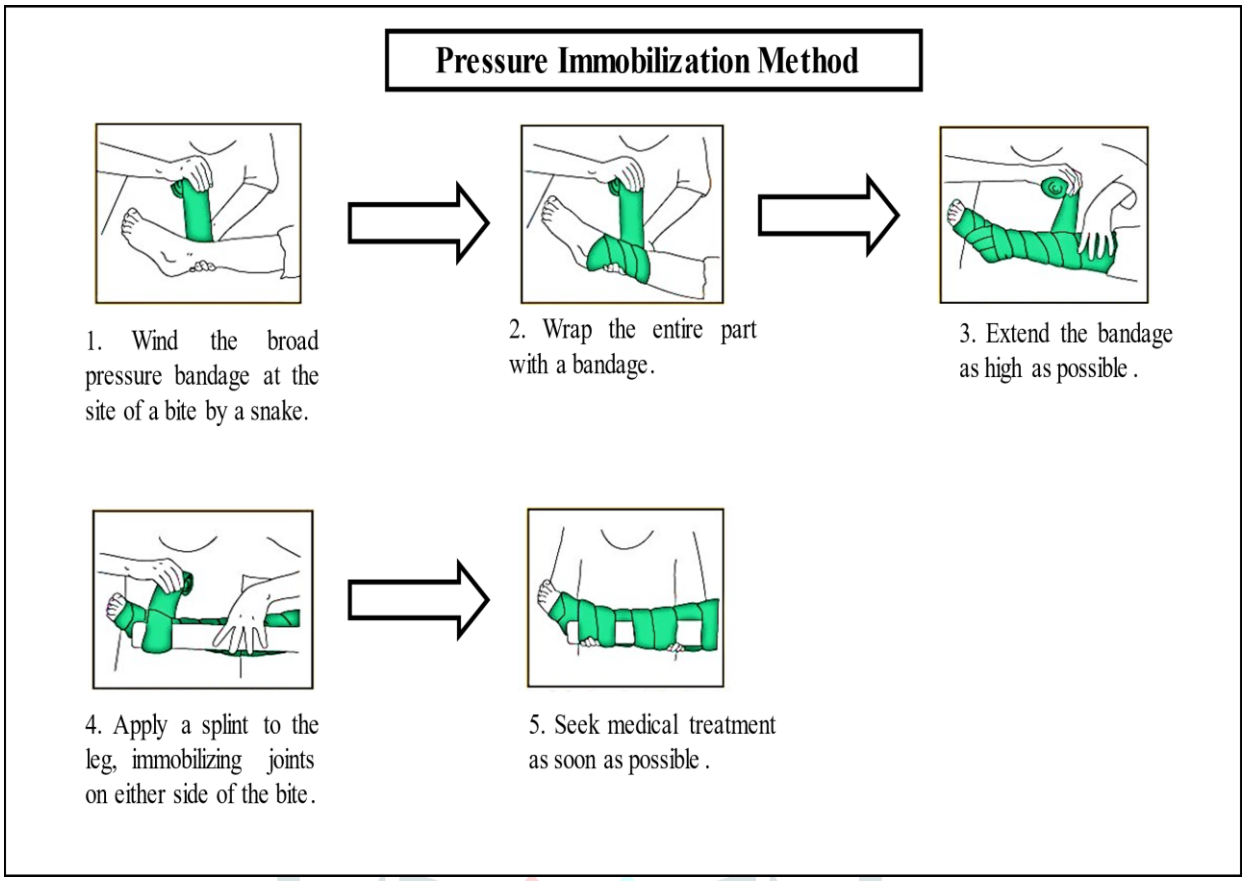
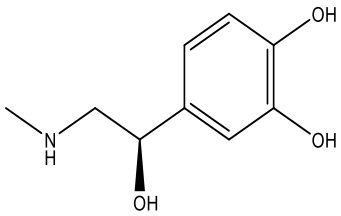
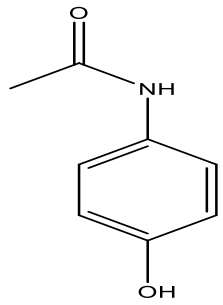
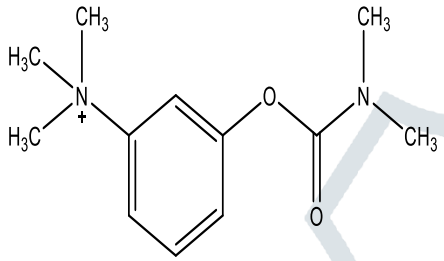
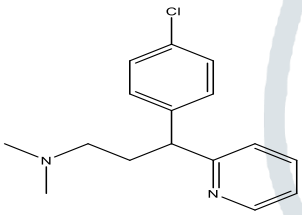
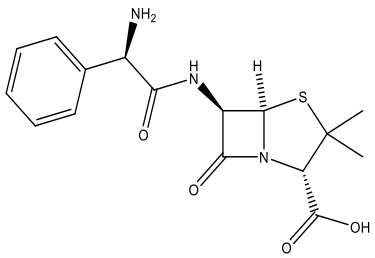
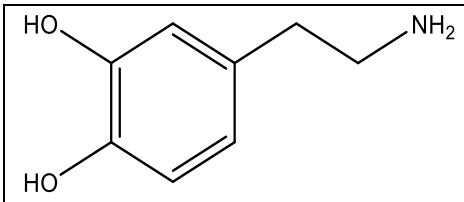


Fig. 5: Pressure Immobilization Method

8. APPROVED DRUGS FOR SUPPORTIVE CARE OF PATIENTS WITH SNAKEBITE ENVENOMING

Drug	Indication	Comment
Adrenaline  (*R*)-4-(1-hydroxy-2-(methylamino)ethyl)benzene-1,2-diol	Treatment of early auto pharmacological anaphylactic reactions due to envenoming or acquired venom hypersensitivity Prevention and treatment of early anaphylactic antivenom reactions	Prophylactic: subcutaneous treatment with a low dose before antivenom treatment Route Of Administration: intramuscular injection

Analgesic  *N*-(4-hydroxyphenyl)acetamide	Routine analgesia Helps to minimize inflammatory reactions.	Aspirin should not be given because of the bleeding risk
Acetylcholinesterase Inhibitor  3-((dimethylcarbamoyl)oxy)-*N*,*N*,*N*-trimethylbenzenaminium	Prolong the biological half-life of acetylcholine at peripheral neuromuscular junctions. Beneficial in neurotoxic envenoming, that act postsynaptically.	Administered after a positive result of a test dose of short-acting edrophonium (a reversible acetylcholinesterase inhibitor).
Antihistamine  3-(4-chlorophenyl)-*N*,*N*-dimethyl-3-(pyridin-2-yl)propan-1-amine	Early anaphylactic reactions (after adrenaline) to antivenom (intravenous administration) Mild, late serum-sickness-type antivenom reactions (oral or parenteral administration)	Ineffective for prophylaxis or severe anaphylaxis
Antibiotics  (2*S*,5*R*,6*R*)-6-((*R*)-2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	Control interference with other microbial infections.	-
Vasopressor Drug	Severe anaphylaxis is refractory to adrenaline	Preferable to excessive fluid replacement,

 4-(2-aminoethyl)benzene-1,2-diol	and fluid volume repletion.	which may precipitate volume-overload pulmonary edema.

9. PREVENTION

The most effective method of preventing snakebites is through education directed at high-risk communities and designed and driven from within those communities. A full range of media should be used, including radio, TV, mobile phone apps, social media, posters, puppet and drama performances, and village-based public meetings. Awareness of snakebite envenoming must be increased, together with advice on safer walking, working, and sleeping. Transport of individuals with a snakebite to clinics where they can receive medical care can be improved, even in areas that are inaccessible to conventional ambulances, for example, using boats or volunteer village-based motorcyclists. Wasting time visiting traditional therapists should be tactfully but firmly discouraged.

❖ ANTIVENOM

Snake antivenom (also known as antivenin, antivenene, and anti-snake bite serum) is the concentrated enzyme-refined immunoglobulin of animals, usually horses or sheep, that have been exposed to venom. It is the only specific treatment currently available for the management of snakebite envenoming and has proved effective against many of the lethal and damaging effects of venoms. The most widely used antivenoms are F(ab′)2-equine polyspecific antivenoms, raised against the venoms of many poisonous snakes. In the management of snakebites, the most important clinical decision is whether to give antivenom, because only some patients need it, it can produce severe adverse reactions, and it is expensive and often in short supply.

Antivenom is most effective by intravenous injection. The range of venoms neutralized by an antivenom is usually stated in the package insert. If the biting species of snake is known, the appropriate monospecific antivenom should be used. In countries in which several species produce similar signs, snakebite victims are treated with polyspecific antivenom, which contains a lower concentration of specific antibodies to each species than the monospecific antivenom.

Antivenom is the only effective specific antidote for the systemic effects of snakebite envenoming. Antivenom comprises concentrated immunoglobulins of horses, sheep, or other large, domesticated animals such as camels that have been hyper-immunized with one or more venoms over periods of months to years. Worldwide, most antivenom manufacturers refine the whole IgG extracted from the animals' plasma by enzyme digestion with pepsin to produce F(ab´) fragments, under the assumption that removal of the Fc moiety from the antigen-binding (Fab) fragment reduces the risk of adverse reactions. Other manufacturers use papain to produce smaller Fab

fragments to improve safety and increase the speed of distribution throughout the body, but with the disadvantage of rapid renal clearance of the antivenom recurrent envenoming becomes a problem. Some antivenoms comprise whole IgG molecules that are usually purified by caprylic acid precipitation. Antivenom antibodies can be extracted by affinity column purification, increasing safety but also cost.

- **PREPARATION OF ANTIVENOM :**

Over 100000 people die a year because of snakebites. Most of the deaths are due to
the lack of proper treatment. Anti-venom is the only antidote that works effectively against the venom. Making antivenom is a resource-intensive, painstaking and time-consuming process. It's not much different now than when it was first created in the 1890s by a protege of Louis Pasteur named Albert Calmette, who was living in present-day Vietnam when a flood forced monocled 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 antivenom. The process is much improved a century later, but the steps remain largely the same.

Step 1: Milking the venom.

The first step is getting your hands on a lot of snakes that are quarantined and monitored for weeks, to months to ensure their good health. Before milking, put on protective gloves. With some of the deadliest snakes, like banded kraits or black mambas, experts often use a short-acting anesthetic to calm the snake down. Take a vial and cover it with a rubber or plastic film. Then, snake in hand, push the fangs through the plastic. Gently squeeze the glands to get out all the venom. To get enough venom, each snake must be milked many times.

Step 2: Cooling down the Venom.

After milking, the venom must be cooled to below minus 20 Celsius and usually freeze-dried for easier storage and transport. Freeze-drying concentrates the venom and removes the water. It's important to clearly label the venom with the snake's species, any relevant subspecies, and geographical origin, since venom can vary wildly between members of the same species, especially between young and old snakes.

Step 3: Immunising.

Horses are most chosen as the animals to create antibodies, because they thrive in many environments worldwide, have a large body mass, friendly, and are easy to work with. Prep the venom for injection by carefully measuring it out and mixing it with distilled water or a buffer solution. Then mix in adjuvant–a chemical that causes the horse's immune system to react and produce antibodies that bind to and neutralize the venom. Inject a small amount of the solution beneath the horse's skin, preferably on its rump or the back of its neck where lymph nodes

and immune cells reside. It's usually a good idea to break up the shot into smaller doses in various locations to avoid causing an ulcer or sore on the skin and to maximize the surface area for an immune reaction. If the horse tolerates the injection, you'll probably give it several more doses days or weeks apart.

Antibodies in the horse's bloodstream peak after about eight to 10 weeks. At that point, the horse is ready to be bled, which involves drawing 3 to 6 liters of blood from the jugular vein.

Step 4: Purifying.

A centrifuge is used to filter the plasma, the liquid portion of the blood not including blood cells. Now it's time to separate the antivenom. This separation begins by getting rid of unwanted proteins. You do this by causing them to precipitate, or fall out, often by adjusting the plasma's pH or adding salts to the solution. One of the last steps involves using an enzyme to break down the antibody into small parts and isolating its active ingredient. After all this effort, your antivenom still must be deemed safe and effective by the FDA, which can take another 10 years.

Step 5: Human application.

After the approval, the purified antibody product is freeze-dried or concentrated into powder or liquid form and put into vials for shipment. Once the product reaches an emergency room and a snakebite victim arrives, the vials are usually filled with saline solution and injected intravenously. If everything goes right, the antibodies then bind to and neutralize the venom, while the liver or kidneys clear out the excess chemicals. A snakebite that needs antivenom requires an average of 20 to 25 vials. Which costs about 25000 dollars for the entire treatment.

CONCLUSION

Snake venoms, considered to be one of the most important bioresources, include pharmacologically active molecules. To better understand the diversity of biological actions of snake venoms and propose new treatment for some pathologies, many studies focused on purification and characterization of new bioactive compounds from venoms. Currently, biomolecules of snake venoms are of great fundamental diagnostic and therapeutic interest. Characterization, biological properties establishment of these bioactive molecules and investigation on their mechanisms, may lead to their eventual use for therapeutic purposes. Therapeutically, proteinases, disintegrins and C type lectins from snake venoms are widely used as anticoagulants or/and anti-platelets. Furthermore, they are valuable tools for understanding the different mechanisms of hemostasis and are also used in the diagnosis of dysfunctions related to coagulation factors such as enzyme activity in thrombin-like venoms that are used for the fibrinogenopathy screening.

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