

SNAKE ENVENOMATION & NOVEL THERAPEUTICS

Abstract

Snakes are poikilothermic vertebrates. Snake venom is recognized as one of the most complex toxins found in the world. Comprising a multifaceted blend of peptides, polypeptides, enzymes, glycoproteins, and various other components, snake venom exhibits the potential for a wide range of toxicological impacts. Snakebite envenomation remains a significant global health issue, particularly in regions where venomous snake populations are prevalent. In recent years, the field of nanomedicine has emerged as a promising avenue for overcoming these challenges. This chapter explores the potential of nanoparticles as a novel therapeutic strategy for neutralizing snake venom in humans. The concept revolves around utilizing engineered nanoparticles as delivery vehicles for venom-neutralizing agents. These nanoparticles can be functionalized with specific targeting ligands to ensure efficient localization at the site of venom injection. Furthermore, the nanoparticles can be designed to encapsulate or conjugate venom-neutralizing compounds, such as small molecules, peptides, or antibodies, enhancing their stability, bioavailability, and therapeutic efficacy. This chapter outlines the current state of research in nanoparticle-based snakebite treatment, including various nanoparticle formulations, venom-neutralizing agents, and preclinical studies. It also highlights the potential challenges and considerations associated with this approach, such as nanoparticle toxicity, immunogenicity, and regulatory approval. In conclusion, the application of nanoparticles for neutralizing snake venom in humans holds great promise for addressing the limitations of current snakebite treatments.

Keywords: nanoparticles, snake envenomation, experimental models

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I. INTRODUCTION

- 1. Definition and Composition of Snake Venom:** Snakes are poikilothermic vertebrates. Snake venom is a mixture of bioactive proteins and peptides produced and secreted by specialized glands in venomous snakes. It is primarily used as a means of predation and defense against potential threats. The snake venom composition varies significantly among diverse species of venomous snakes [1]. Typically, venom contains enzymes, neurotoxins, cardiotoxins, cytotoxins, and other molecules that target specific physiological systems in prey or predators. These venom components work in synergy to immobilize or incapacitate the snake's prey, making it easier for the snake to consume its meal [2]. While the exact composition varies, most venomous snake venoms contain a combination of these toxic compounds tailored to their ecological niche.
- 2. Overview of Venomous Snake Species:** There are numerous species of venomous snakes found across the globe, belonging to different families and genera. Some of the most well-known venomous snake families include Elapidae (e.g., cobras, kraits, coral snakes) and Viperidae (e.g., vipers, pit vipers, rattlesnakes). Each family and species within it has evolved unique venom compositions and delivery mechanisms, making their study a diverse and intriguing field of research. Common venomous snakes found in India are cobra (*Naja naja*), common krait (*Bungarus caeruleus*), Russell's viper (*Daboia russeli*) and saw scaled viper (*Echis carinatus*) [3, 4].
- 3. Geographic Distribution of Venomous Snakes:** Venomous snakes can be found in a wide range of habitats, including deserts, rainforests, grasslands, and even some aquatic environments. Their distribution is influenced by factors such as climate, prey availability, and territorial boundaries. Different venomous snake species are native to specific regions around the world, with some species confined to certain continents or countries, while others may have broader distributions [1].
- 4. Importance of Studying Snake Venom and Its Potential Applications:** The study of snake venom and its components has profound implications for biomedical research and drug development. Venom toxins have evolved to target specific molecular pathways, making them valuable tools for understanding cellular processes and signaling cascades. As a result, researchers have increasingly focused on the potential applications of snake venom in various fields of medicine. Some key areas of interest include:
 - Development of Antivenoms:** Snakebite envenomation is a significant global health issue, affecting millions of people annually. It affects mainly the cardiovascular, nervous, renal and respiratory systems. Understanding the composition and action of snake venom is crucial for developing effective antivenom treatments to save lives and mitigate the impact of snakebites [5].

Venom from various sources offers exciting potential for medical applications. Components in venom possess analgesic properties, showing promise for novel pain-relieving drugs in chronic conditions. In cancer research, venom toxins display the ability to target cancer cells and hinder tumor growth, opening new avenues for drug development. Venom neurotoxins offer insights into neural signaling pathways, aiding neuroscience research and potential therapies for neurological disorders.

Additionally, certain venom peptides have vasodilatory and antihypertensive effects, offering opportunities for developing cardiovascular drugs. Ongoing research on snake venom components continues to hold promise for novel drugs and therapies in various medical fields [5, 6].

- 5. Detection of Snake Venom:** The identification of snake venom holds significant forensic value, serving to establish the precise cause of death and prevent erroneous assertions. Various techniques have been devised for snake venom detection, including enzyme-linked immunosorbent assay (ELISA) and optical immune assay (OIA) [7]. In tropical countries the setting up of regional forensic science laboratories with snake venom detection facilities and capacity building is essential for addressing the mortality and morbidity due to snake envenomation.

II. VENOMOUS SNAKE SPECIES AND THEIR VENOMS

- 1. Major Families of Venomous Snakes:** Venomous snakes belong to different families, each with its distinct characteristics and geographical distribution. Some of the major families of venomous snakes include, Elapidae, Viperidae, Atractaspididae & Colubridae [8].
- 2. Characteristics of Venomous Snake Venoms:** Snake venom features various components, including enzymes like proteases, phospholipases, hyaluronidases, and L-amino acid oxidases. These contribute to tissue breakdown, blood clotting interference, and cellular effects. In Elapidae family snake venoms, neurotoxic components affect the nervous system, leading to paralysis or neuromuscular dysfunction. Viper venoms typically contain hemotoxic and cytotoxic elements causing damage to blood cells, vessels, cell death, and tissue necrosis. Additionally, some snake venoms have cardiotoxic effects on the cardiovascular system. Venom potency varies among species, with differing levels of envenomation severity and rapidity [5, 6].
- 3. Variation in Venom Composition among Species:** Venom composition varies significantly among snake species, driven by their adaptation to specific ecological niches and prey. Several factors influence venom composition, including prey preference. Snakes targeting small vertebrates often have neurotoxin-rich venoms for rapid immobilization, while those consuming larger prey may possess cytotoxic and hemotoxic components for efficient digestion. Geographic distribution and regional prey availability further impact venom composition. Phylogenetic differences among snake families contribute to distinct venom compositions, and even within the same species, inter-species variation can exist. This understanding is crucial for developing tailored antivenoms and exploring potential biomedical applications of venom components [8, 9].

III. COMPONENTS OF SNAKE VENOM

Snake venoms contain a wide array of bioactive molecules, each with specific effects on the prey or potential predators [5, 10]. These components play critical roles in immobilizing or incapacitating the snake's target. Below, we'll explore the major components found in snake venom:

1. Enzymes:

- **Proteases:** Proteases are enzymes that break down proteins by cleaving peptide bonds. In snake venom, metalloproteases and serine proteases are common. Prominent constituents in the venoms of Crotalid and Viperid snakes are metalloproteases which are involved in disrupting the extracellular matrix and basement membranes, leading to tissue damage and facilitating the spread of venom in the victim's body. Serine proteases interfere with blood clotting mechanisms, leading to the disruption of hemostasis [11]. Snake Venom Metalloproteinases (SVMPs) exhibit diverse activities which encompass hemorrhagic, fibrinolytic, prothrombin activation, blood coagulation factor X activation, apoptotic, platelet aggregation inhibition, pro-inflammatory, and blood serine proteinase inhibitor inactivation. The SVMPs exhibit multiple functions beyond their renowned hemorrhagic activity [12].
- **Phospholipases:** Phospholipases are enzymes that hydrolyze phospholipids in cell membranes. Snake venom PLA(2)s, found in abundance, serve both toxic and digestive roles, exhibiting a wide array of effects, including neurotoxicity, myotoxicity, hemolysis, edematogenicity, hyperalgesia, inflammation, hypotension, platelet aggregation inhibition, anticoagulation, cytotoxicity, and bactericidal activity [13]. These enzymes, known as phospholipase A2s (PLA2s), are prevalent in snake venoms from Colubridae, Elapidae, and Viperidae families. They primarily target phospholipids with unsaturated fatty acid tails at the sn-position, yielding lysophospholipids and unsaturated fatty acids. The resultant hydrolysis products modify cell membrane properties, initiating downstream signal transduction pathways, thus inducing widespread cellular pathology [14].
- **Hyaluronidases:** Hyaluronidases are enzymes that degrade hyaluronic acid, an important component of the extracellular matrix. By breaking down this matrix, hyaluronidases facilitate the diffusion and spread of venom through tissues, increasing its local and systemic effects. Snake venom hyaluronidases (SVHYA) play a significant role in tissue destruction during envenomations and are known as spreading factors due to their ability to enhance venom toxin delivery. Interestingly, SVHYA act on hyaluronic acid (HA), producing low molecular weight HA fragments (LMW-HA). LMW-HA generated acts as a damage-associated molecular pattern, recognized by Toll-like receptors 2 and 4, triggering cell signaling cascades that lead to innate and adaptive immune responses. These responses involve the generation of lipid mediators, production of interleukins, upregulation of chemokines, activation of dendritic cells, and proliferation of T cells [15].
- **L-amino Acid Oxidases:** L-amino acid oxidases (LAAO) are involved in various biological activities, such as promoting inflammation and apoptosis in target cells. Snake venoms contain significant concentrations of L-amino acid oxidases (LAAOs), which vary according to each snake species, potentially contributing to venom toxicity. LAAOs display catalytic specificity for long chain hydrophobic and aromatic amino acids and exhibit activity across a wide pH and temperature range. Their structures, molecular masses, and isoelectric points show considerable diversity. LAAOs have the ability to modulate platelet function, leading to local effects on

plasma clotting disorders and other related effects. Additionally, LAAOs can induce apoptosis in various cell lines and demonstrate antimicrobial and antiparasitic activity [16].

- 2. Neurotoxins:** Snake venom toxins have traditionally been categorized into two types of neuromuscular blockade: pre-synaptic and post-synaptic. Pre-synaptically active neurotoxins, mainly neurotoxic phospholipase A2 toxins (PLA2s), bind to motor nerve terminals, causing depletion of synaptic ACh vesicles, impaired ACh release, and later, motor nerve terminal degeneration. This leads to a three-phase neuromuscular block, involving immediate ACh release depression, followed by enhanced release, and eventually complete inhibition of NMJ transmission. Post-synaptically active neurotoxins, known as alpha-neurotoxins, bind to post-synaptic muscle nAChRs and belong to the group of "three-finger toxins" (3FTXs) characterized by a shared toxin structure resembling three outstretched fingers. These alpha-neurotoxins are classified into three main groups: long-chain, short-chain, and non-conventional alpha-neurotoxins. They produce a reversible, non-depolarizing post-synaptic block by competitively inhibiting ACh binding to muscle nAChRs, leading to a curare-mimetic neurotoxic effect. However, recent insights into neuromuscular transmission and descriptions of different neurotoxicity patterns suggest that this view may be oversimplistic and requires reevaluation [17].
- 3. Cytotoxins:** Cytotoxins (CTs), also known as cardiotoxins, are toxins present in cobra venom that exhibit a three-finger (TF) fold. These CTs are approximately 60-residue-long peptides, containing up to 4 disulfide bonds. The β -strands originate from the hydrophobic core formed by the disulfides, taking the shape of the three loops, which gives the fold its name. In contrast to neurotoxins (NTs), another group of TF proteins from snake venom that exert their effects through specific interactions with protein receptors, CTs do not have a specific protein target identified. Unlike NTs, CTs are amphiphilic and possess cytotoxic properties against various cell types, including cancer cells [18].
- 4. Other Bioactive Molecules:** Snake venoms contain a diverse array of bioactive molecules with specific effects. Some examples include nucleotidases, enzymes that degrade nucleotides and release purine and pyrimidine derivatives with various biological activities. Metalloproteinases contribute to tissue damage and inflammation by degrading extracellular matrix proteins. Additionally, the venom may contain biogenic amines like serotonin and histamine, causing vasodilation, increased vascular permeability, and contributing to inflammatory responses. Furthermore, natriuretic peptides in the venom have diuretic and vasodilatory effects on the cardiovascular system [19].

IV. MECHANISM OF ACTION OF SNAKE VENOM

- 1. Pathophysiology of Envenomation:** The pathophysiology of snake envenomation involves the complex interactions of venom components with various physiological systems in the victim's body. Different venom components target specific molecular pathways, leading to a wide range of local and systemic effects [20]. Common pathophysiological mechanisms include:

- **Local Tissue Damage:** Proteases, phospholipases, and cytotoxins in snake venom contribute to local tissue damage and inflammation at the site of the bite. These components degrade extracellular matrix proteins and cell membranes, leading to swelling, pain, and tissue necrosis [21]. Venom metalloproteinases exhibit diverse domain structures, including metalloproteinase domains, disintegrin-like and high cysteine domains, and lectin-like subunits. These zinc-dependent enzymes share similar zinc binding environments. Some directly induce hemorrhage by selectively cleaving basement membrane bonds, disrupting endothelial cell interactions and causing cellular alterations linked to hemodynamic factors. This results in endothelial gaps and extravasation. Beyond hemorrhage, these metalloproteinases cause skeletal muscle damage and myonecrosis due to ischemia from bleeding and reduced perfusion. Due to their pivotal role in tissue damage, they're valuable targets for natural and synthetic inhibitors, which could complement antivenom treatments to neutralize their effects [22].
- **Hemostatic Disturbances:** Venomous snake procoagulant toxins categorically induce consumption coagulopathy, potentially leading to spontaneous or uncontrolled bleeding as clotting cascade factors are depleted. The impact on clotting factors varies among different snake venoms. These toxins, like metalloproteinases, play a pivotal role in activating prothrombin, factor V, factor X, or thrombin-like enzymes (fibrinogenases), making them significant procoagulant agents [23]. Thrombotic microangiopathy, often accompanying venom-induced consumption coagulopathy, manifests as thrombocytopenia, microangiopathic hemolytic anemia, and acute kidney injury [24]. The nature and extent of consumption coagulopathy vary based on the specific procoagulant toxin. These toxins typically activate clotting factors, resulting in diminished fibrinogen levels post-envenoming. Thrombin-like enzymes or fibrinogenases typically cleave the α -chain or β -chain of fibrinogen, yielding fibrinopeptide A or B. This leads to fibrinogen consumption without fibrin formation [25].
- **Neurotoxic Effects:** Neurotoxins, particularly α -neurotoxins in Elapidae venoms, target acetylcholine receptors at neuromuscular junctions resulting in the blockade of neuromuscular transmission and paralysis of muscles, including those involved in respiration. Snake venoms often contain neurotoxins inducing a descending flaccid neuromuscular paralysis, which can involve dangerous bulbar and respiratory muscle blockade. Two primary neurotoxin types are α -neurotoxins and β -neurotoxins.

α -Neurotoxins, part of the three-finger toxin family, act postsynaptically at neuromuscular junctions, binding to cholinergic receptors and causing flaccid paralysis by inhibiting acetylcholine binding [26]. Conversely, β -neurotoxins are usually PLA₂s acting presynaptically. For instance, β -bungarotoxin from kraits binds voltage-gated potassium channels, leading to enzymatic phospholipid hydrolysis, neurotoxicity, vesicle fusion, and calcium influx, ultimately destroying nerve terminals and causing prolonged paralysis. Some neurotoxic PLA₂s also act intracellularly, exacerbating degenerative effects [27].

Other venom neurotoxins include dendrotoxins and fasciculins in African mamba venoms, blocking potassium channels and inhibiting acetylcholinesterase, respectively. Combined actions lead to excitatory effects and muscle contractions. Additionally, certain cysteine-rich secretory proteins induce smooth muscle paralysis [28].

- **Cardiovascular Effects:** Cardiotoxins and certain venom enzymes can impact the cardiovascular system, leading to changes in heart rate, blood pressure, and cardiac function, varying from mild disruptions to severe complications. Venom proteins and peptides can have diverse effects, including both harmful and protective impacts on the heart. Notable categories of these compounds include cobra cardiotoxins, phospholipases A₂, natriuretic peptides, and bradykinin-potentiating peptides. Additionally, there is a group of proteins that promote angiogenesis, such as vascular endothelial growth factors, which exhibit blood pressure-lowering and heart-protective properties. These venomous elements with cardiotropic and vasoactive properties hold promise as candidates for the development of innovative drugs aimed at preventing or mitigating pathological processes in cardiovascular diseases, a leading global cause of mortality [29].
 - **Systemic Toxicity:** Certain snake venom components, including metalloproteinases and cytotoxic molecules, can enter the bloodstream, resulting in widespread effects on various organs and tissues. These effects encompass weakness, fatigue, anxiety, fluctuating heart rate, rapid breathing, sweating, gastrointestinal symptoms, temperature disturbances, swelling of lymph nodes, metallic taste, headache, thirst, respiratory issues, low blood pressure, collapse, shock, cerebral oxygen deficiency leading to altered mental states, and anaphylactic reactions. Rapid-onset low blood pressure within 2 hours indicates severe intoxication, which may be transient, persistent, or life-threatening. Kidney problems manifest as protein, hemoglobin, and myoglobin in the urine, elevated waste products in the blood, and reduced urine production. Seizures can occur due to oxygen deprivation rather than direct venom neurotoxicity. Electrocardiograms (ECGs) may display specific changes, and hematological issues range from coagulation problems to bleeding from various sites. Lab tests reveal abnormal clotting times, decreased clotting factors, low platelet count, and elevated D-dimer levels. Neurological symptoms are specific to certain snakebites and typically emerge within 8 hours following a latent period [30, 31].
- 2. Interactions with Cellular Components:** Snake venom components engage with various cellular elements in the victim's body, yielding diverse effects. Phospholipases in venom target cell membranes, causing membrane disruption and lysis. Neurotoxins bind to specific nerve cell receptors, altering signal transmission and causing paralysis. Hemotoxic elements, including proteases, influence platelets and blood cells, inducing coagulation disruptions and hemolysis[32].

V. NEUTRALIZATION OF SNAKE VENOM

1. Antivenom Development:

- **Production and Purification of Antivenoms:** Snake antivenoms are required to adhere to the contemporary standards of identity, purity, safety, and efficacy outlined by the current Good Manufacturing Practices (GMPs) governing modern biopharmaceutical drugs. The industrial production of snake antivenoms involves multiple key phases: 1) creating reference venom pools, 2) generating hyperimmune plasma, 3) purifying antivenom immunoglobulins, 4) formulating the antivenom, 5) stabilizing the formulation, and 6) conducting quality control assessments for both in-process and final products. This review provides an overview of the prevalent technology employed in the industrial manufacturing of snake antivenoms [33].

Antivenoms are developed by immunizing large animals, such as horses or sheep, with small, controlled amounts of snake venom. The immune response in these animals results in the production of polyclonal antibodies against venom toxins. These antibodies are then harvested from the animal's blood, purified, and formulated into antivenom products. The production and purification process are complex and require careful consideration of factors such as antigen selection, animal welfare, and immunogenicity. Furthermore, antivenoms must be thoroughly tested for safety, potency, and efficacy to ensure their effectiveness in neutralizing venom toxins in snakebite victims [34]. Antivenoms are produced through the plasma fractionation of immunized animals, resulting in products containing complete IgG or immunoglobulin fragments such as F(ab')₂ or Fab. Antivenom formulations exhibit distinct physicochemical attributes across different laboratories, encompassing variations in total protein content, levels of protein aggregates and non-IgG plasma proteins, and the inclusion of Fc fragments in whole IgGs [35].

To mitigate the drawbacks inherent in traditional antivenom manufacturing, a number of researchers have explored alternative approaches using existing production methods. They have investigated the use of recombinant or synthetic toxins, peptides, and DNA vaccination strategies to generate antibodies with therapeutic relevance through immunization techniques. This advancement not only eliminates the current necessity for venom in antivenom production, thereby obviating the need for maintaining collections of venomous animals, but also significantly reduces the labor-intensive and potentially hazardous aspects associated with animal handling for personnel [36]. Nonetheless, venom remains essential for quality control and research validation of antivenom, ensuring the efficacy of newly manufactured antivenom products.

Challenges and Advancements in Antivenom Development:

Antivenom development encounters challenges such as venom complexity, regional variations in snake populations, high production costs, storage demands, and potential adverse reactions [35]. Researchers have explored alternatives like recombinant antibodies or synthetic peptides for safer, more effective antivenoms.

Monoclonal immunoglobulins (IgGs) target medically significant snake species (*Naja* spp., *Crotalus* spp., *Echis* spp., *Laticauda* spp., and *Bothrops* spp.) and specific venom components (phospholipase A₂, metalloproteinase, thrombin-like) [37].

Chinese Hamster Ovary (CHO) cell systems mimic human glycosylation patterns and are favored for large-scale antibody production. The conventional CHO cell method uses the fed-batch process with subsequent batch harvest. Some industries adopt efficient continuous chromatographic techniques like Simulated Moving Bed Chromatography (SMBC) for higher purified product output [38].

2. Nanotechnology for Neutralizing Snake Venom: Nanoparticles serve as effective carriers for antivenom, enhancing stability and bioavailability. They optimize sustained release, improving antivenom's binding to toxins, increasing potency, and reducing dosage. Portable nanoparticle-based formulations enable rapid on-site treatment in remote areas. They also stabilize antivenom during storage and transportation, extending shelf life and ensuring efficacy [39].

Earlier research has demonstrated the remarkable stability of metal nanoparticles and their suitability for snake venom inhibition [40, 41]. Silver nanoparticles (SNPs) are synthesized by chemical reduction method and characterized using UV-Visible spectroscopy, Dynamic Light Scattering (DLS) and Transmission electron microscope (TEM) [42]. DLS study showed the formation of complex between SNPs and crude viper venom with decrease in hydrodynamic size of complex compared to the size of native viper venom. Venom components might have adsorbed on the surface of SNPs and making Viper venom proteins more compact in nature results in modification of its activity [42]. Scientists from the University of Calcutta's toxicology laboratory attached gold nanoparticles (GNP) to HMBA (2-hydroxy-4-methoxy-benzoic acid), a compound derived from the root of the Anantamul (*Hemidesmus indicus*) herb, known for its anti-venom properties. Through animal trials, it was demonstrated that the GNP-HMBA pairing effectively counteracted various forms of toxicity (including kidney, muscle, and liver toxicity) in mice exposed to Russell's viper venom, which is among the most potent snake venoms [40].

Synthetic polymer nanoparticles have also been used as broad spectrum antivenom. This versatile antivenom consists of polymer nanoparticles (NPs) specially designed to capture the primary protein toxins found in elapid snake venoms. These durable, economically efficient NPs can be subcutaneously administered directly at the bite site immediately after envenomation, effectively curbing or minimizing local tissue damage and curtailing the systemic dissemination of toxins following envenomation [43].

The potential immunoadjuvant properties of chitosan nanoparticles (CNPs) loaded with venoms from *B. jararaca* and *B. erythromelas* in the production of sera targeting these venoms has been explored. Stable CNPs were created through ionic gelation, and mice were subcutaneously immunized over six weeks using 100 μ L of each snake venom at concentrations of 5.0% or 10.0% (w/w), either encapsulated in CNPs or combined with aluminum hydroxide (AH). Assessment of protein interactions with CNPs demonstrated their capacity to induce antibody levels comparable to those induced by AH, even with lower antigen doses. Furthermore, CNPs exhibited reduced inflammatory effects due to their controlled protein release. This suggests that CNPs offer a promising avenue for delivering peptides and proteins from snake venoms and hold potential for the development of novel vaccines [44].

The increasing adoption of environmentally friendly approaches has spurred the creation of silver nanoparticles (AgNPs) through various sources, including bacteria, fungi, algae, and plants, enabling extensive production while minimizing contamination referred to as 'Green Synthesis'. Green synthesis represents an eco-conscious and biologically compatible method, typically involving the utilization of a capping agent or stabilizer (to regulate size and prevent clustering), plant extracts, yeast, or bacteria to achieve these objectives [45]. Rhizomes of the Indian male fern, *Dryopteris cochleata*, were employed for the green synthesis of silver nanoparticles with the goal of enhancing the bioactivity of the plant extract and assessing the inhibitory effect on *N. naja* snake venom. The findings from the neutralization experiments demonstrated that the silver nanoparticles synthesized from *D. cochleata* effectively reduced tissue damage, leading to a substantial inhibition of phospholipase A2 and the venom of *N. naja* snakes [46].

Recent approaches in snake venom neutralization with a focus on nanoparticles hold immense promise for revolutionizing snakebite management. These innovations aim to enhance the efficacy, accessibility, and safety of antivenom therapy, ultimately improving the prognosis for snakebite victims. While challenges remain, such as regulatory approval and scalability, the integration of nanotechnology into snakebite treatment represents a significant stride towards more effective and accessible care for those affected by venomous snakebites.

- 3. Small Molecule Inhibitors:** Small molecules are being investigated as inhibitors of venom toxins. These compounds can interfere with the toxic mechanisms of snake venom and provide a novel approach to managing snake envenomation. Compound 5d, a small molecule derived from apigenin, has demonstrated its efficacy against SVMs both in computational simulations and live experiments. Apigenin based small molecules with a flexible substitution at second position of the chroman moiety are prepared by multi-component Ugi reaction using various aromatic amines, t-butyl-isocyanide and halo acetic acids. A library of various apigenin analogues was synthesized, and among these, compound 5d exhibited a significant dose-dependent reduction in Echi carinatus (EC) venom-induced local hemorrhage, tissue necrosis, and myotoxicity [47].
- 4. Gene Therapy:** Gene-based therapies, including DNA vaccines, are being explored to prompt the production of specific antivenom antibodies within the victim's body, offering rapid, on-demand protection against venom toxins. This approach leverages the cost-effective and swift insights gained from random gene sequencing, known as expressed sequence tags (ESTs), into the intricate biochemical processes underlying various biological phenomena. This is exemplified in identifying clinically significant toxins in snake venoms, aiding immunotherapeutic treatments for snakebites. Snake venoms typically contain over a hundred proteins with significant inter- and intraspecific diversity in toxicity, posing challenges for purification and characterization. A systematic method for selecting immunoprotective sequences based on molecular sequence data alone can lead to the development of innovative, highly avid polyspecific antivenoms, enhancing dosages' effectiveness and minimizing toxicity in snakebite victims. DNA immunization offers a rational approach to toxin-specific immunotherapies, as previously demonstrated, inducing antibody levels and protective responses suitable for antivenom production and cross-reactivity with venoms from diverse viper species and genera [48].

VI. FUTURE PERSPECTIVES

Progress in snake venom therapeutics involves tailored treatments based on genomics and proteomics, comprehensive understanding through multi-omics integration, efficient nanoparticle delivery systems, global collaboration for development and distribution, streamlined regulatory processes, community engagement, and healthcare professional training in snakebite-prone regions.

VII. CONCLUSION

In conclusion, the field of snake venom research has made significant strides in recent years, offering novel perspectives on the treatment and mitigation of snakebite envenomation. From the development of innovative antivenom delivery systems, such as nanoparticles, to the utilization of molecular sequencing data to design toxin-specific immunotherapies, these advancements hold great promise in improving the outcomes for snakebite victims. By leveraging green synthesis methods and exploring natural compounds like apigenin derivatives, researchers are expanding the toolkit for combating venomous snakebites. These approaches not only enhance antivenom efficacy but also contribute to reducing the burden of snakebite-related morbidity and mortality, particularly in regions where access to conventional antivenom may be limited. As the global scientific community continues to collaborate and innovate, the prospects for more effective and accessible snakebite treatments are brighter than ever, offering hope to those at risk of snake envenomation worldwide.

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