ORIGINAL ARTICLE

A Review on Anti-Thrombotic Agents Derived from Snake Venom Protein

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ABSTRACT

Background: Snakes have always attracted scientists and it caused awe and fear because of the harmful strength of its toxins and the components of various toxins from one snake to another. In 2017, the World Health Organization (WHO) listed snake venom as a neglected high-priority disease, with snakes causing up to 2.7 million venomous bites, nearly 100,000 victims, and nearly three times the number of human disabilities.

Objective: i- Snake venom background and therapeutics properties ii- The effects of snake bite iii- the mechanism of haemostasis iv- Types of antithrombotic agents derived from snake venom proteins.

Conclusion: Snake venoms are a group of diverse compounds and because of their diversity, they lead to many effects that have different effects on the body, causing an imbalance and mastery of the victim. Just as these components have toxic effects, they have clinical and therapeutic effects. The previous research shown the clinical benefits of snake venom, such as the drug captopril used to treat high blood pressure, And the defibrase used for the prevention of thrombotic disease. Therefore, snake venom components need several studies. They are still unidentified due to the difficulty of obtaining them adequate.

Keywords: Snake venoms, antithrombotic, haemostasis, venom proteins.

INTRODUCTION

Snakes have always been a source of attraction to scientists [1]. Moreover, it caused awe and fear not only because of their elegant movement of its limbs but also because of the harmful strength of its toxins and the components of various toxins from one snake to another [2]. In 2017, the World Health Organization (WHO) listed snake venom as a neglected high-priority disease, with snakes causing up to 2.7 million venomous bites, nearly 100,000 victims, and nearly three times the number of human disabilities [3,4]. Snake venoms are a huge mixture of different protein components, each protein in it acting on different functional activities on a set of varied physiological goals [5]. These venoms vary extensively between and within snake species [6]. Snakebite can cause many hazardous pathologies related to neurotoxic, cytotoxic, and hemotoxic effects of the venom [6,7]. Both (Table.1 and Figure.1) demonstrates the most famous types of poisonous snakes responsible for the largest mortality in the world [8]. The diagnosis of poisoning is a pure clinical skill with no diagnostic tool kit available yet [9].

Table 1: The most poisonous snakes.

Snake	Common Name	Part in Figure1	Family	Country
Echis Ocellatus	West African Saw-Scaled Viper	B	Viperidae	West African
Bitis Arietans	Puff Adder	C	Viperidae	Savannah and Grasslands from Morocco, Western Arabia and Africa Except For The Sahara and Rainforest Regions
Naja Naja	Cobra	D	Elapidae	India, Pakistan, Bangladesh, Sri Lanka, Nepal, And Bhutan
Bungarus Caeruleus	Common Krait	E	Elapidae	Indian Subcontinent
Daboia Russelii	Western Russell's Viper	F	Viperidae	Asia (Common in India)
Bothrops Atrox	Common Lancehead	G	Viperidae	The Tropical Lowlands of North of the Americas East Of the Andes
Bothrops Asper	Terciopelo	H	Viperidae	Distribution from Southern Mexico To Northern South America.
Oxyuranus Scutellatus	Papuan Taipan	I	Elapidae	North & East Australia and New Guinea island.

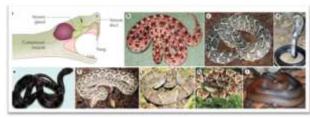This table shows the most poisonous snakes (Family and Country) [10].

Figure 1: Venomous snakes. This figure shows the Schematic illustration of the venom system in a snake of the family Viperidae and the most poisonous snakes [10].

When snakes inject their venom, they use their tusks from the anterior part of maxillary bones (Figure.1) [11,12]. Depending on how big the canines are, the toxin is injected under the skin or intramuscularly [13]. The toxins exert local effects on adjacent tissues once they are delivered, while others spread uniformly through the lymphatic system and blood vessels., allowing the toxins to act in several organs (Figure. 2) [14]. Therefore, we notice here that the spread of poison in the body causes poisoning in many organs, and every poisoning that occurs is caused by a component of snake venom [15].
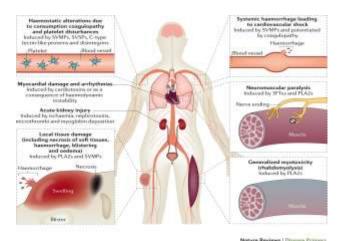

Figure 2: snake bite.

This figure shows the Action of snake venom toxins on different body systems [10,20]. Multiple changes can occur after a snake bite such as local swelling, local necrosis, non-specific early

systemic symptoms (vomiting, headache, abdominal pain, critical diarrhoea, and fall with unrecordable blood pressure), spontaneous haemorrhage, neurotoxic effect (dizziness, headache, and fainting), myotoxic effect (myopathic tumours in skeletal muscle), cardiac effect (rapid pulse, low blood pressure and shock), renal failure [16,17,18,19,20].

Snakes have multiple types of venom glands, which are nine species, including a family Elapidae and Viperidae [21]. The composition of the snake venom protein may change according to types of family and class, and this change affects the effectiveness of the protein and its strength [6]. Snake venom consists of four groups of proteins [20,24]. The first major protein group present in most types of venoms namely. The second group commonly exists but to a less amount than the first group: Kunitz peptides (KUN), L-amino acid oxidases (LAAO), Disintegrin (DIS), C-type lectins (CTL), Cysteine-Rich Secretory Proteins (CRiSP), and Natriuretic peptides (NP) [5,6,10]. Last group is rare: Galactose-binding proteins, aminopeptidases or warping, and Cobra venom factors (CVF) [5,6,10]. Moreover, not all proteins present in all snakes venomous [5,6,10].

In our research, we will discuss the updated information about the antithrombotic effect of various snake toxins depending on their mechanism of action and therapeutic properties.

METHOD

A review was done by searching the databases as PubMed, google scholar and Medline. Key words included: ''Snake venom'', ''Snake bite'', '' Venom anticoagulant' 'and ''anticoagulant proteins. The updated search period is from 2016 to 2021.

Haemostasis and Thrombosis systemic: Haemostasis is the mechanism in our human body that includes several steps, and this results in what is called a "plug" that closes the damaged site in the blood vessels to protect against loss of blood [16]. Haemostasis can occur in two stages: primary and secondary [16]. Primary haemostasis is aimed to form a weak platelet plug, which is achieved by four steps [16]. Vasoconstriction is the first step occur after damaging of blood vessels and occur within thirty minutes [16,25,26,27,28,29]. Platelet adhesion is the second step, it is a mechanism which platelets start rolling through the blood vessels and attach to the revealed subendothelial vWF and collagen in damaging areas, leading to platelet adherence and closing the injury [16,29]. Platelet activation is the third step, it happens when platelet binds to vWF, its activation passes by two processes [16]. The second is releasing of cytoplasmic granules of platelets [16]. There are two pathways of platelet activation that occur through thrombin [16]. One of them that thrombin stimulates platelets immediately by binding to a protease-activated receptor through proteolytic cleavage [16]. Another way is that thrombin releasing of cytoplasmic granules, including serotonin, thromboxane A2, platelet activation factor, and ADP [16,29]. Binding to the P2Y1 receptor will P2Y1 receptor will aids to platelet aggregation by making conformational [16,29]. Binding to the P2Y12 receptor will aid to induce the coagulation pathway [16]. Platelets produce TXA2, which enhances vasoconstriction and platelet aggregation [16]. As a result, the platelet activation mechanism will make the zone eligible for platelet aggregation [16]. The last step of primary hemostasias is platelet aggregation [16]. When platelet activated the Gp IIb/IIIa receptors will bind to vWF and fibrinogen [16]. Then platelet will start binding to each other and will form a weak platelet plug [16,29]. Finally, primary haemostasis occurs to prevent bleeding temporarily until secondary haemostasis start [16]. Secondary haemostasis is defined as the transformation of fibrinogen into fibrin, which stabilizes the soft weak platelet plug and makes it a hard, insoluble fibrin clot [4]. These processes are achieved by using one of the pathways; the extrinsic or the intrinsic pathway which focus on the activation of FX and then completes their processes by the common pathway [16]. In secondary haemostasis, calcium ions are required for all processes because it plays an important role in all 3 pathways [4]. Both intrinsic and extrinsic pathways are shared to continue coagulation with the common pathway [4]. Thrombosis is the formation of clots in the blood vessels and leads to partial or complete obstruction, and a decrease in the amount of blood flowing in the blood vessels, because of changes in persistent blood components [4,16].
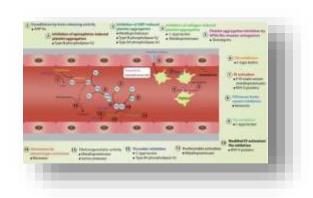

Figure 3: Anticoagulant Mechanism of Snake Venom on coagulation pathway.

This picture displays the paths of coagulation and the mechanism of several components of anticoagulant snake venoms [30].

Enzymatic Anticoagulant Proteins

Phospholipase A2 (PLA2): Phospholipase A2 is a superfamily it is a toxic enzyme in snake venom [30]. It is a small protein and has worked in two bonds: The first bond is sn-2 fatty acyl bond is binding to sn-2 fatty acyl of phospholipids membrane to make lysophospholipids and free fatty acids [30]. The second bond is the sn-2 ester bond PLA2 acts to hydrolyze the sn-2 ester bond of glycerophospholipids is a structural lipid bilayer cellular membrane [31, 32,33]. They main divided for PLA2 into two groups: Old World snakes (group I) and New World snakes (group II) they are different in disulfide bond pattern both two groups have comparable molecular weight (13–15 kDa) [18, 34]. Studies have established the enzyme Phospholipase A2 has an anticoagulant effect by two mechanisms: non-enzymatic and enzymatic [35]. Enzymatic mechanism it is inhibiting the formation of prothrombinase complex (FVa, Ca2+, phospholipids, and FXa) [18]. The non-enzymatic mechanism it is inhibiting the formation of thrombin by inhibiting the FXa (Figure.4) [18,36].
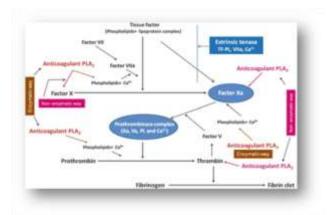

Figure 4: Anticoagulant Mechanism of Phospholipase A2.

This figure shows two mechanisms for Phospholipase A2 as anticoagulant: Non enzymatic and enzymatic [18].

Table 2: The Summary of Metalloproteinases groups.

Groups	Molecular weight	Domain structure	Hemorrhagic effect
P-I	20–30 kDa	Only a proteinase domain	Weak
P-II	30–60 kDa	Proteinase and disintegrin domains	High
P-III	60–100 kDa	Cysteine-rich domain, proteinase domain and disintegrin-like domain	Higher than other groups

This table shows the Metalloproteinases groups based on domain structure and molecular weight and their Haemorrhagic effect [40].

Serine proteases (SVSP): SVSP is a group of enzymes derived from snake venom, and like other components of snake venom, it performs several functions such as platelet aggregation [37], blood coagulation [7,26,30,37,41], fibrinolysis [7,26,30,41], hypotensive [26,37], neurotoxicity [26], and anticoagulant effect [26]. These enzymes are divided into two groups [26]. The first group, which is predominant, is thrombin-like enzymes, and it stimulates the blood clotting process and forms fibrin by cleaving the Aα and/or Bβ chains of fibrinogen [7,26,30,41].

L-amino acid oxidases (LAAO): L-amino acid oxidases (LAAOs) are flavoproteins found in Elapidae and Viperidae, but especially in Crotaline [11,32,42]. These enzymes represent a variety of biological activities in the victim such as cytotoxicity, myotoxicity, and edema, which induce clinical symptoms of envenomation [11, 23,40,43]. L-amino acid oxidases are shown a broad range of pharmacological activities such as antimicrobial, platelet aggregation (anticoagulant), cellular apoptosis (anticancer), and anti-HIV activity [1,44]. L-amino acid oxidases (LAAOs) which activates the stereospecific de-lamination of the L-amino acid layer into alpha-ketogenic acid, which produces ammonia and hydrogen peroxide (Figure.5) [32,33,40,45,46]. These enzymes make up one to nine percent of total venom proteins [32,36,40]. While some research has shown that LAAOs have a suppressive effect on platelets, others have shown the opposite [36,40]. H2O2 generation and the subsequent synthesis of thromboxane A2 cause platelet aggregation [13,36,40]. Although four techniques have been shown to generate LAAO's inhibitory action [23]. Platelets exposed to H2O2 have reduced ADP attachment [40], (3) collagen, thrombin, ADP, and arachidonic acid [40], (4) FIX activity is selectively suppressed [36]. According to some research, they also exhibit de-aggregation effects on rabbit and human platelets [40].
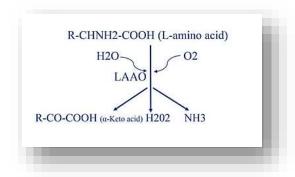

Figure 5: Dissecting the Enzymatic Mechanism of L-Amino Acid Oxidases.

The conversion of a L-amino acid into an alpha-keto acid, which results in the formation of ammonia and hydrogen peroxide, is depicted below [23].

Nucleotidases: Their primary function is to release adenosine by hydrolyse 5'-nucleotides to nucleosides which exhibit antiplatelet and hypotensive effect [6]. Also, adenosine contributes to biodistribution of toxins, immobilization, increase vascular permeability, inhibit neurotransmitter release, that leads to sedation, bradycardia, hypotension, and locomotor depression [6]. These changes can enhance synergistically the anticoagulant action of certain toxins as ADPases, phospholipases A2 and Disintegrins [6]. Another study found that Nucleotidases show antiplatelet effect by interact with blood FIX in coagulation cascade [37,47].

Non-Enzymatic Anticoagulant Proteins

Three-finger toxins (3FTx): The core is clamped by four preserved disulphide bridges forming a three-finger shape (Figure.6) [27, 37,41,48]. The functional multiplicity of 3FTxs through diversity in amino acid sequences and other structural changes, that leads to bind to different receptors and exhibit a wide variety of biological effects such as neurotoxic, cytotoxic, cardiotoxic effects, and hypotensive effect [3,22,27,31,37,41,48]. 3-FTxs is one of the biggest family of snake venom toxins, more than 800 sequences of 3FTxs that have been hoarded due to the diversity in amino acid sequences and other structural changes [3,27]. One of its many interesting functions is antiplatelet and anticoagulation [27,48]. Where more than one toxin substance extracted from 3ftx has been reported, it has an anti-platelet or anticoagulant effect [27,48]. In this table (table.3) we can see the names of the toxic substances, their Origin, and their mechanism of action [27,48,49,50,51,52].
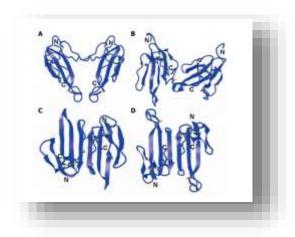

Figure 6: The Structure of Three-Finger Toxin.

This figure shows the variation of structure lead to variety of biological effects; (A) Homodimer of α-cobratoxin. (B) Irditoxin. (C) 0Haditoxin. (D) κ-bungarotoxin. (N) &(C) N- and C-terminus, yellow color expresses bisulfide bonds [48].

Table 3: Antiplatelet and Anticoagulant of Three-Finger Toxins.

3FTx	Origin	Mechanism of Action
Anti-Platelet		
Dendroaspin	Dendroaspis Jamesoni Kaimosae	Inhibits ADP-mediated platelet aggregation and inhibits binding of the purified alpha-IIb/beta-3 platelet fibrinogen receptor (ITGA2B/ITGB3) to paralyzed fibrinogen.
S5C1		
Thrombostatin	Dendroaspis Angusticeps	
γ-bungarotoxin	Bungarus Multicinctus	
TA-bm16		
NTL2		
KT-6.9	Naja Kaouthia	• Inhibit inductive ADP, thrombin, and arachidonic acid. • Hinder platelet aggregation by adenosine diphosphate. Inhibit platelet induced through the

		P2Y12 receptor.
Anti-Coagulant		
Hemextin AB complex	Hemachatus Haemachatus	• Links to FVIIa only Inhibition of ETC formation by Prevent interaction between FVII and TF
Exactin		• Inhibition of ETC formation. Links to FVIIa and TF complex that interfere with the FX link.
Ringhalexin		
Najalexin	Naja Atra	
Ophiolexin	Ophiophagus Hannah	

This table shows the names of Antiplatelet and Anticoagulant of Three-Finger Toxins, their origin, and their Mechanism of action [27,48].

Kunitz peptides (KUN): Kunitz-Type Proteinase Inhibitors was the first protease inhibitor was discovered in snake venoms1972 by Takahashi and colleagues, it contains 60 amino acid, 6 cysteine residues and stabilize by 3 disulphide bridges [6,18]. KUN was singled out from the venom of D. russelli and reported to exist in Elapidae and Viperidae snake venoms [6]. This family of protease inhibitors has structural similarity to bovine pancreatic trypsin inhibitor BPTI, which is responsible for serine proteases inhibition such as activated FX, elastase, trypsin, thrombin, and chymotrypsin [6,9]. It has a wide range of biological activities like ion channels blockage especially voltage-gated potassium channels which may lead to anticoagulation, fibrinolysis, and inflammation [6, 53].

C-type lectins (CTL): C-type lectins are non-enzymatic proteins and Ca2+ dependent carbohydrate proteins [42-49]. These family of proteins are found in many different tissue types and are primarily involved in adherence and immunity and signalling related to inflammation [18]. All these family proteins portion homology in their carbohydrate-recognition domains (CRDs) [18,49], Though, all these family has C-type lectins and high difference in amino acid sequence so-called C-type lectin fold [18]. Platelets are an integral part of the haemostat [18]. The first step of the constitutes blood coagulation cascade is Platelet aggregation [18]. C-type lectins bind to multiple integrins and receptor to each block or inhibit platelet aggregation: von Willebrand Factor, Thrombin, GPIb (receptor glycoprotein Ib), FIX and/or FX [14,17,18,34]. C-type lectins binding to von Willebrand Factor to form complex to inhibit\prevent activation of platelet aggregation [14,34]. Another mechanism is binding to receptor GPIb (is platelet membrane glycoprotein Ib) and inhibits the formation of thrombosis [14,18]. Binding with FX and/or FIX formation complex to act as anticoagulants [34].

Disintegrins (DIS): Disintegrins bind to integrins through several methods:1/Arginine-glycine-aspartate (RGD)-containing peptides resulting in an active site that modulates the integrin activity [12,40].; 2/It inhibit ADP-induced platelet aggregation [40].; 3/Acts as αIIbβ3 antagonists because it is playing a big role in platelet functions, haemostasis, and arterial thrombosis [24,28].

CONCLUSION

Snake venoms are a group of diverse compounds and because of their diversity, they lead to many effects that have different effects on the body, causing an imbalance and mastery of the victim [6]. On the other hand, snake venom is also considered to have clinical benefits that have been shown by previous research, as there are currently many drugs that have been extracted from snake venom, such as the drug captopril used around the world, which inhibits the angiotensin-converting enzyme (ACE) to treat high blood pressure [37] , And also the defibrase is a product based on batroxobin which is a procoagulant SVSP that extracted from snake venom of Bothrops atrox and used for the prevention of thrombotic disease [30]

Just as these components have toxic effects, they have clinical and therapeutic effects [6]. Therefore, snake venom components need several studies [6]. They are still unidentified due to the difficulty of obtaining them adequately [6]. In this research we gathered information about the effect of snake venom on the blood.

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