CHAPTER II

REVIEW OF LITERATURE

2.1 Biochemical and proteomic characterization of some medically essential scorpions around the world

Scorpions are one of the deadliest venomous animals found all over the globe including India. They are accountable for more than 1.2 million stings annually resulting in more than 3250 deaths in a year [1; 2; 3]. During the last several years, the field of proteomics has evolved considerably with the power of 1D and 2D SDS-PAGE, multidimensional chromatographic techniques (gel filtration chromatography, ion exchange chromatography, RP-HPLC) to separate complex mixtures of proteins and demonstrated the advantages of application of LC/MALDI-TOF-MS or LC/ES-MS in the identification of different venom components [4; 5; 6]. These are the methods for de-complexion of venom protein before LC/MS-MS analysis which are prerequisites for better resolution and identification of more proteins.

One of the most significant dynamic pharmacological components of scorpion venom is peptides [7]. Biologically active scorpion venom proteins/ peptides are classified into two groups: disulfide bridge peptide (DBP) and non-sulfide bridge peptide (NDBP). The disulfide bridge peptide (DBP) are neurotoxic and specifically impairing with various ion channels, whereas non-sulfide connect peptides (NDBP)s have appeared to have bradykinin–potentiating, antimicrobial, haemolytic, cell flagging and immuno-modulating activities [8; 9; 10]. From the LC-MS/MS analysis of various scorpion venom proteomes, it was found that Na^+ and K^+ channel inhibitors are predominated in the majority of the scorpion venom proteomes [4; 11].

The last decade has seen an expansion of research techniques utilized to identify, characterize, and quantify the venom composition of venomous animals. Traditional approaches have relied on biochemical analyses of venom enzymes and venom profiling by SDS PAGE and gel filtration chromatography. However, more recently, these techniques have been coupled with high throughput genomic, transcriptomic, and proteomics approaches to provide a more profound and comprehensive analysis of a specific venom [12; 13; 14; 15; 16; 17; 18]. Several studies have also drawn a good correlation between venom composition with toxicity and pathophysiology of sting [19; 20; 21].

Different scorpion families such as Buthidae, Scorpionidae, Urodacidae, and Hemiscorpiidae comprise different types of venom components which may also have therapeutic implications [22]. For example, venom components from *Buthus martensii Karsch* show analgesic effects [23], anticancer properties [24], immunomodulatory properties [25], antimicrobial effects etc. [26]. Likewise, different venom components from the Indian black scorpion (*Heterometrus benganelsis*) shows ant-proliferative and anti-apoptotic effect [27]. One component named bradykinin-potentiating peptide from Brazilian scorpion *Tityus serrulatus*, increases the hypotensive action of bradykinin [28]; Hyaluronidase was also isolated from its venom [29]. Different scorpion species such as *Tityus sp.*(Brazil), *Buthus martensii* (China, Korea), *Centruroides noxius* (Mexico) show an effect on the immune system (release histamine, TNF-α, interleukins etc.) that have a role on inflammation [30]. A low molecular weight component (adenosine and dipeptides LeuTrp and IleTrp) from *Heterometrus laoticus* (from Vietnam) scorpion venom is responsible for anticoagulant activity [31].

Mesobuthus martensii is one of the most populous scorpions in eastern Asian countries. The venom proteome of this species was characterized by 2DE, SDS-PAGE and HPLC applications where 134 proteins were identified comprised of 43 typical toxins and 7 atypical toxins (including 3 Na^+ channel toxins, 3 K^+ channel toxins and 1 no-annotation toxin) 72 cell-associated proteins and 12 venom enzymes. This venom proteome is most abundantly comprised of lower molecular massproteins (around 10 kDa). *M. martensii* crude venom contained three novel Na^+ channel toxin sequences: comp201_c0_seq1_3, comp162_c0_seq1_6 and MMa37864. Only 29.58% of comp201_c0_seq1_3 511 sequence identity was found with neurotoxin 8 (Amm VIII) in in public database searches, which was a *Androctonus mauretanicus* derived long-chain (4 C-C) α-Na^+ channel toxin [32]. Comp162_c0_seq1_6 showed a maximum similarity of 32.35% (mature peptide) with *Androctonus crassicauda* derived peptide, acra3 (a β-Na^+ channel toxin) [33]. MMa37864 possessed a identity of 30.43% with toxin Tx273 (β-Na^+ channel toxin) isolated from the *Buthus occitanus israelis.* The poor similarity among these three peptides and the other recognised scorpion toxins led researchers to hypothesise that they might be categorised as novel Na^+ channel toxins [11].

Proteomic analysis of *Androctonus bicolour* by LC–MS/MS analysis also revealed 16 various venom peptides which include ion channel toxins and some antimicrobial peptides [34]. One of the deadliest scorpions in existence is *Buthus occitanus* (*B.*

occitanus). By enabling a global perspective of the structural elements of such complex matrices, top-down and bottom-up proteomic analyses are implemented to ease screening. The nano-high liquid chromatography coupled with nano-electrospray tandem mass spectrometry (nano-LC-ESI MS/MS) was used in conjunction with top-down and bottom-up strategies. From the mass spectrometry analysis, it was found that *B. occitanus* venom contains 200 molecular masses ranging from 1868 to 16,720 Da and among them, the most representative venom peptides were between 5000 and 8000 Da. Interestingly, combined top-down and bottom-up LC-MS/MS results showed the finding of several toxins, preferably ion channels toxins which target the ion channels, including Na^+ (NaScTxs), K^+ (KScTxs), Ca^{2+} (CaScTx) and Cl^- (ClScTxs) channel toxins, amphipathic peptides, antimicrobial peptides (AMPs), hypothetical secreted proteins and myotropic neuropeptides. This investigation reveals the molecular diversity of *B. occitanus* scorpion venom and determines components that could be pharmacologically active [35].

The Colombian scorpion *Tityus pachyurus* is fetal and has potential to cause deadly incidents to humans. From mass spectrometry analysis of *T. pachyuru*, 104 distinct compounds were identified. A strong Shaker B K^+-channel (shaker B K^+-channel is a type of ion channel) blocker was discovered during electrophysiological experiments using heterologously produced ion channels in Sf9 cells. This peptide, which goes by the moniker Tpa1, has a molecular mass of 2457 atomic mass units and consists of 23 amino acid residues that are tightly bound together by three disulfide bridges. It is the third member of subfamily, and -KTx13.3 has been suggested as its systematic name. The peptide has the descriptive name Tpa1 and has a molecular mass of 2457 atomic mass units. It has 23 amino acid residues that are tightly packed together by three disulfide bridges. The systematic name for it is suggested to be -KTx13.3; it is the third member of subfamily. The *in vivo* experiment with mice model convincingly demonstrated that these venom peptides had harmful consequences [36]. One of them is Tpa2, stabilized by four disulfide bridges and it contains 65 amino acid residues and a molecular mass of 7522.5 atomic mass units. Similar to the β-scorpion toxins, it was found to alter the Na^+-currents of the F-11 and TE671 cells in culture. These findings show that toxic peptides are present in *T. pachyurus* venom and support the notion that encounters with this species of scorpion pose a significant risk to people in Colombia [36].

Among 86 poisonous species of scorpions found in India, *M. tamulus* is the most lethal with reported fatalities mostly in children and adults [37]. There are reports on the characterization of active fractions from the scorpion venom *M. tamulus* that act on various ion channels. Several toxins, for example, Iberiotoxin, a blocker of high conductance Ca^{2+-} activated K^+ channel [38]; tamapin, inhibitor of small conductance Ca^{2+}-activated K^+ channel [39]; lepidopteran-selective toxin (BtTx3 (3,796 Da) and ButaIT (3,856.7 Da)), act as insecticidal agents against lepidopteran insect species [38; 40] have been isolated from *M. tamulus* venom (MTV). This venom also contains potent cardiopulmonary toxinscausing pulmonary oedema [41]. Hyaluronidase isolated and purified from various scorpion venoms such as *Palamneus gravimanus, Heterometrus fulvipes, Tityus serrulatus, Buthus marthesi* is necessary for the spread and absorption of venom's toxic components [42; 43; 29; 44].

2.2 Pharmacological targets of scorpion venom toxins

2.2.1 Na^+ and K^+channel toxins: pathophysiology and mechanism of action

Animals, plants, and bacteria all contain ion channels, which control the flow of ions across cell membranes [45]. These channels participate in the generation of membrane potentials, signal transduction, the release of neurotransmitters, contraction of muscles, release of hormones, sensation of physical and chemical stimuli, motility, and growth of cells [46; 47]. They can be divided into groups based on their homologous sequence, ion selectivity, and gating mechanisms for both opening and closing. There are three types of gating channels: a) Voltage-gated channels, b) ligand-dependent channels, and c) Channels with mechanical sensitivity [48]. Voltage-gated Na^+, K^+, and Ca^{2+} channels all have similar structures in mammals and are typically made up of a pore-forming subunit, or subunit.

Peptide toxins, which regulate voltage-gated Na^+/K^+ channel activity, are prevalent in the venom of scorpions. In K^+ channels, the selectivity filter is made up of the conserved signature sequence TVGYG in the P-loop [49; 50], while four amino acid residues (DEKA) found in an analogous location in each of the domains of Na^+ channels regulate selectivity for the Na^+ ion in those channels [51]. Several different voltage-gated ion channel types are involved in the action potential, the electrical signal produced by nerve cells [52]. Our understanding of the action potential is based on the analysis of the squid axon [53], in which voltage-gated Na^+ (Nav) channels open for a short period before

rapidly deactivating; after a short while, K^+ channels are activated and remain open for a longer interval [54; 52]. Na^+ ions influx into the cell and K^+ ions efflux to the extracellular environment as a result of the action potential travelling on depolarization. Action potentials serve a variety of purposes in the neuron cell bodies and axons, and different types of neurons have unique action potential patterns as well (Fig. 2.1) [52].

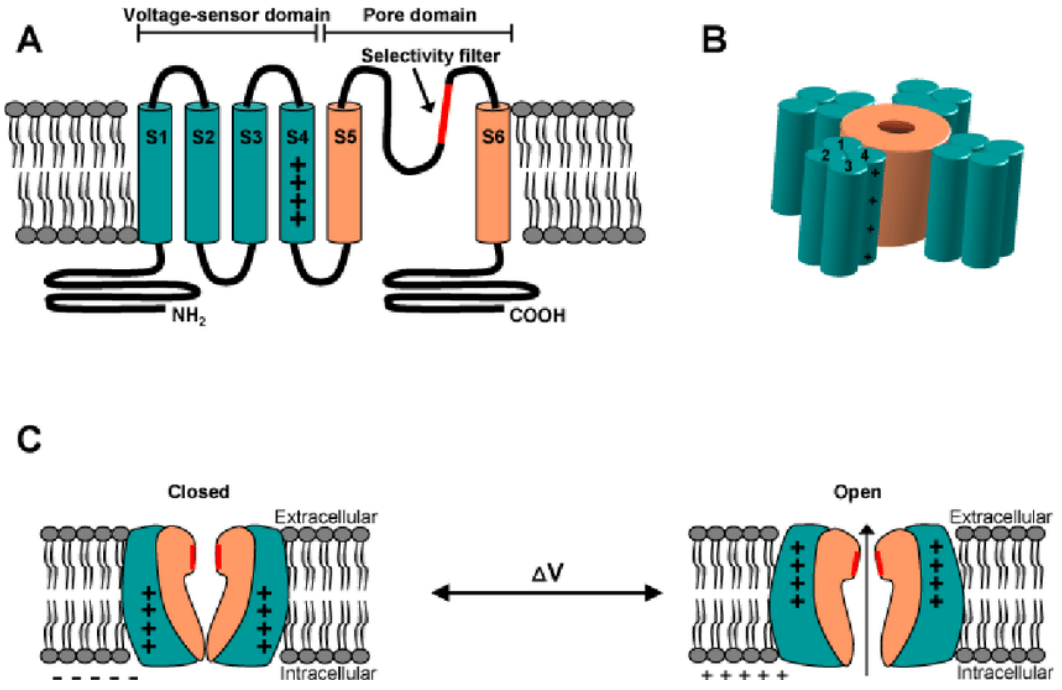

Fig. 2.1. General architecture of a voltage-gated ion channel. (A) Each subunit is made up of six transmembrane helices (S1-S6 lined with intracellular N and C termini). (B) The four sub-units tetramerize to shape an ion channel with a pore-forming central unit (orange) enclosed by four VSDs (green). (C) S4 charges move in an outward direction withan alteration in membrane voltage that leads to the ion channel opening [49].

The toxins found in scorpion venom are similar in structure and have comparable physicochemical properties, but they have distinct pharmacological effects that have evolved over millions of years as a result of evolution and natural selection, favouring the animals with neurotoxins that can block or regulate ion channels [55; 56]. The primary mechanism causing the pharmacological effects of neurotoxins is their interaction with ion channels [56]. Regarding the Buthidae family, whose neurotoxins are extremely toxic and exhibit variation in their affinity for ion channels found in mammals and arthropods,

as well as within and between species, it is possible that peptides acting on ion channels may play a fundamental and functional role in envenomation during evolution. This is particularly relevant concerning scorpions, which share a common ancestor with them [57].

2.2.1.1 K^+ channel blockers and mechanism of action

The four primary categories of K^+ channels are as follows: voltage-gated, tandem pore domain, inwardly rectifying, and calcium triggered. Although these channels have similar structures, the distinctions between these types primarily relate to how the gate receives its signal [50]. The subunit in K^+ channels is made up of the tetramerization of four distinct domains and thus clustered to form a pathway for the ion-permeation across the membrane [58; 59]. Each domain of the subunit is made up of helical segments with six transmembrane (TM) (S1-S6) [60], and are further divided into a pore-forming domain (PD), which consists of S5 and S6 segments, and a voltage-sensing domain (VSD), which is made up of segments S1 through S4 and has positively charged residues in segment S4 [58; 59]. Four VSDs are located throughout each of the four PDs that are clustered together in the pore.

The intracellular activation gate, which is found at the intersection of the four S6 helices, prevents the entrance of ions when it is closed or deactivated and is opened and closed by the VSD [58; 59] (Fig. 2.2). K^+ channels are distinguished by two transmembrane helices and a short loop between them known as the P LOOP. K^+ channels all share this same canonical structure, known as 2TM/P, which consists of two inner helices and a loop. After changes in membrane potential, the S4 segment moves outward or inward, causing conformational changes that, in turn, cause the channel pore to open or close, as appropriate [51]. The domains' pore-lining loops (P-loops), which connect S5 and S6, include the selectivity filter and is made up of conserved residues specific to each channel, enables selectivity for particular ions [58].

"K^+ channel toxins" are scorpion toxins that inhibit various K^+ channels and have been extensively studied [61]. They are shorter than Nav toxins but are closely related to them [7]. K^+ channel-specific toxins (KTx), which have been exploited in the structural and functional characterisation of several K^+ channels, are abundant in scorpion venom. KTx has been divided into four families: α-, β-, γ- and κ-KTx based on the primary amino acid sequences and cysteine pairing [62; 63]. Previous studies demonstrated that many KTxs

from *M. eupeus* and *M. martensii* scorpions were isolated which block different K^+ channels [64] such as maurotoxin acting on the Kv1.2 channel [65], BeKm-1 block hERG channel [66; 65], Kunitz-type toxins block the Kv1.3 channel [67], charybdotoxin acting on the BKCa channel [68], and BmP05 block the SKCa3 channel [69; 70]. Additionally, several active components of scorpion venom that affact Ca^{2+} and Cl^- channels have been identified [71; 72; 73; 74].

Pore-blocking peptides bind tightly to the outer vestibule of the K^+ channel, obstruct the ion channel's selectivity filter, and prevent K^+ ion transit [75; 76]. The conformational changes in the channel protein determine the mechanism of K^+ channel activity. At the resting membrane potential, the ion channel is shut and does not conduct ions. The voltage sensor is impacted by increased membrane potential, and this results in the channel opening [77]. The ion channel responds to any slight shift in membrane potential due to the high sensitivity of the voltage sensor, and the open channel carries ions until it reaches the inactivation phase [78]. K^+ channel inactivation causes by two mechanisms: (1) N-type and (2) C-type (Fig. 2.2). The channel remains in an open conformational state during the N-type of inactivation, but the pore is blocked by the N terminal of the α-subunit of the K^+ channel. In the N-type mechanism, when the N terminal fragment is removed, the inactivation is abolished, and when the N terminal fragment is reinserted as a peptide, the inactivation is restored. However, the N terminal is not implicated in the C-type of inactivation. This sort of inactivation is caused by structural components located in the vestibule of the selectivity filter. in the C-type of inactivation, the N terminal is not involved [78].

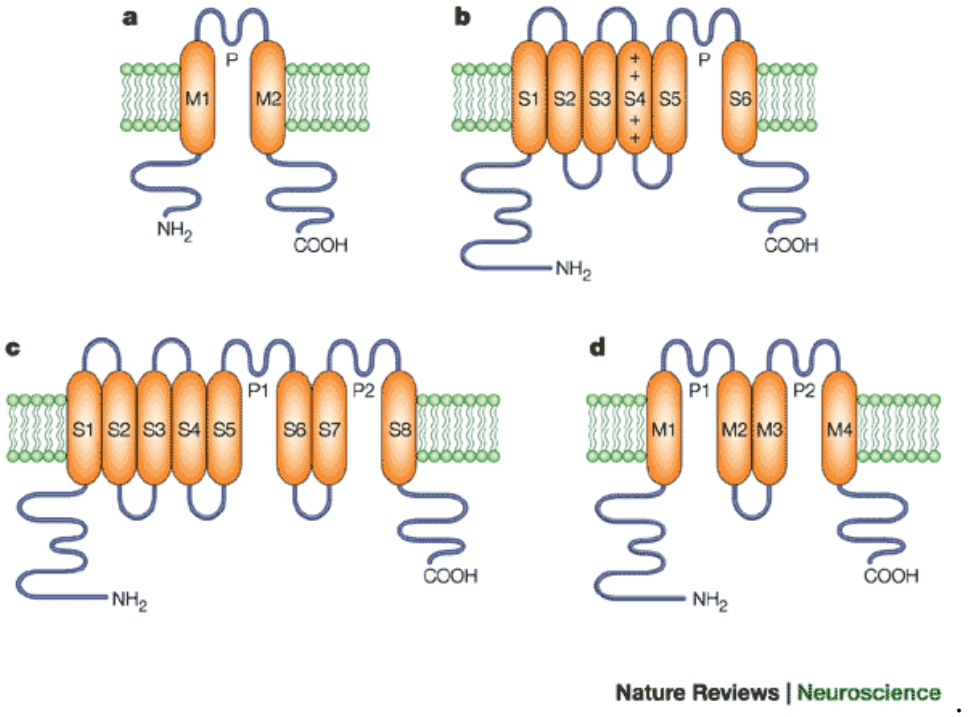

Fig. 2.2. The four primary classes of K^+ channels. a | 2TM/P channels, b | 6TM/P channels, c | 8TM/2P channels, d | 4TM/2P channels. The presence of positive signs on S4 denotes its function in voltage sensing in voltage-gated K^+ channels [49].

2.2.1.2 Na^+ channel blockers and mechanism of neurotoxin binding with Na^+ channel

Voltage-gated Na^+ channels (VGSCs) are responsible for the rapid input of Na^+ ions that raises the action potential in muscle, nerve, and endocrine cells [8]. The body's excitable cells (muscles, neurons, and endocrine cells) are all distributed with VGSC isoforms, each of which is associated with distinct characteristics in the relevant cells and tissues. Nav 1.5 and 1.4 are significantly expressed in the cardiac muscle and skeletal muscle, respectively, while Nav 1.9, 1.8, and 1.7 isoforms are present in the peripheral nervous system. The central nervous system contains Nav 1.6, 1.3, 1.2, and 1.1 isoforms [79]. The term "Nav channel long chain toxin" refers to highly lethal toxins that alter the voltage-gated Na^+ channel (Nav) [80; 81; 82; 9]. According to Rodriguez et al. (2005)[9] and Cao et al. (2014) [83], the Na^+ toxin (BmKIM) from scorpion *M. martensii* inhibited Na^+ currents in rat ganglion neurons and myocytes and stopped cardiac arrhythmia in a mouse model [84].

Na^+ channels being transmembrane complexes consist of two subunits: the large core protein is α-subunit (220–260 kDa), and it is associated with a different small regulatory unit i.e β-subunit (22–36 kDa). The pore containing α-subunit is selectively permeable to Na^+ ions, which are composed of four homologous domains (DI–DIV). All of which include six transmembrane segments (S1–S6) [66]. Three cytoplasmic loops connect these four domains to create a bell-shaped protein (Fig 2.3). The voltage sensing module generated by S1-4 is the first module found in each of the four domains (DI-DIV), and the second module is the pore-producing module developed by S5, S6, and the connecting loop [85]. There are two ways in which toxins affect VGSCs. When the neurotoxin physically blocks the pore and reduces Na^+ ion conductance, it either causes a blockage of the pore or a modification of the gating that changes the voltage dependence and gating kinetics of the ion channels. The first method is used by toxins when they interact with site 1. For instance, site 1 pore blockers include tetrodotoxin (TTX) and saxitoxin (STX). Site 2 toxins like grayanotoxin and batrachotoxin block inactivation, causing channels to stay continuously active [79]. Toxins from sea anemones and scorpions bind to site 3 and prevent inactivation [72]. Site 4 toxins like those seen in scorpions and spiders cause the activation to become hyperpolarized [86]. When associating with VGSC, site 5 toxins such as ciguatoxins and brevetoxins show a noticeable effect, such as activation inhibition and a voltage-dependent activation shift towards hyperpolarization. Finally, through blocking inactivation, δ-conotoxins interact with site 6 and have effects that are comparable to those of the neurotoxins that affect site 3 (Fig. 2.3) [79]. Proteases and protease inhibitors are some other components in addition to these neurotoxins.

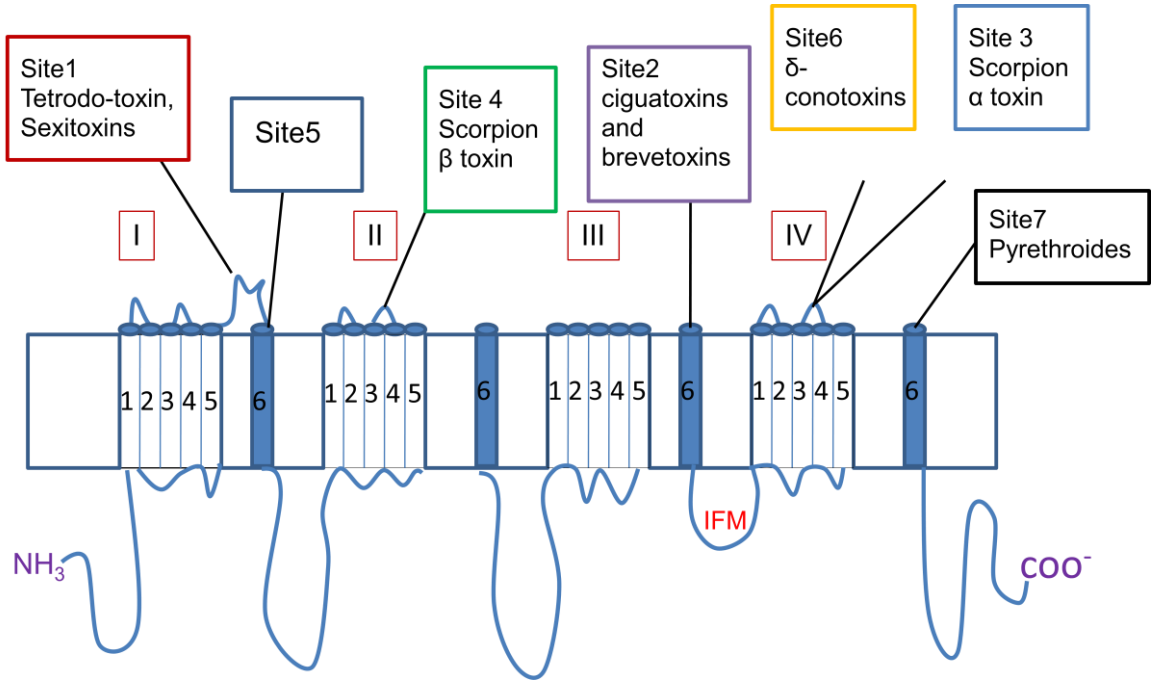

Fig. 2.3. Diagram showing the neurotoxic binding regions on the voltage-gated Na^+ channel's -subunit (VGSC) [79].

2.2.2 Glycaemic response of scorpion venom and administration of insulin

Post-scorpion envenomation induces a severe autonomic storm through the massive release of catecholamines, cortisol, elevated glucagon levels, thyroid hormones, and either suppressed insulin levels or hyperinsulinemia (insulin resistance) [87; 88]. Previously, it was studied that scorpion venom (*Mesobuthus tamulus concanesis*, Pocock) (4 mg/kg) treated in dog models lower the release of insulin when the mode of venom injection was intravenous [88]; whereas insulin secretion become suppressed 30 min after venom injection, and increased insulin levels 60 min post venom injection by subcutaneous mode [89]. Glucose-induced release of insulin become inhibited from the islets' β cells in the endocrine pancreas by adrenalin storms and the abrupt inhibition of insulin secretion leads to hyperglycaemia. Increased levels of the counter-regulatory hormones (glucagon, cortisol, and catecholamines) obstruct the anabolic effects of insulin and cause hyperglycemia through increased glycogenolysis or insulin resistance [90; 91].

2.2.3 Scorpion venom-induced inflammatory response

The inflammatory response is initiated by a cascade involving systems, the release of mediators and cell elements. Balanced cytokine production is essential for health to

maintain homeostasis. Some cytokines are overproduced, leading to disorders with severity ranging from moderate to fatal. A well-adapted anti-inflammatory response may also be able to control local inflammation in the presence of local infection or tissue damage and stop it from spreading to the entire body.

Scorpion venoms can release catecholamines, corticosteroids, bradykinin, and prostaglandins, all of which have been shown to increase the release of immunological mediator cytokines. Sofer in 1995, [92] first studied the involvement of inflammatory response post scorpion stings in humans. The classification of cytokines into pro- and anti-inflammatory responses is crucial for the structural and functional regeneration of damaged tissue, but excessive production of pro-inflammatory signals can exacerbate tissue damage due to the products produced by inflammatory cells [93]. IL-1, IL-6, and TNF are the main pro-inflammatory cytokines that start an efficient defence against external infections. The overproduction of these mediators, however, has the potential to be deleterious and can eventually result in shock, multiple organ failure, and death (Table 2.1) [94; 95]. Contrarily, anti-inflammatory cytokines such as IL-4, IL-5, IL-6, and IL-10 are essential for reducing the exacerbated inflammatory process and maintaining homoeostasis for the proper functioning of vital organs, but an excessive anti-inflammatory response may also suppress body immune function (Table 2.1) [96; 97; 98; 99].

It was reported the release of high levels of IL-6 in mice sera treated with *Centruroides noxius* and *T. serrulatus* scorpion venoms. IL-6 is commonly used as an indicator of systemic pro-inflammatory cytokine activation. Elevated levels of IL-6 were observed in mice sera exposed to *C. noxius* and *T. serrulatus* venoms [100; 101]. IL-6 is often used as a marker for systemic activation of pro-inflammatory cytokines [102]. The Brazilian *T. serrulatus* venom-treated human and mice sera show an increased level of IL-1 mediator and high concentrations of this cytokine were found in the macrophage supernatants of mice exposed to *T. serrulatus* venom [103; 104]. Increased levels of IL-1β were found in the plasma of people who had been moderately or severely stung by *T. serrulatus* [105]. High levels of IL-1 and IL-1 in the serum of mice exposed to the Mexican scorpion *C. noxius* [101].

Table 2.1. Inflammatory mediators involved in scorpion stings.

Scorpion	Cytokines produced	References
Androctonus australis hector Experimental animals (rats)	IL-1β, IL-4, IL-6, IL-10, and TNF-α.	[106]
Buthus martensi Karch	NO and paw oedema	[107]
Centruroides noxius Experimental animal (mice)	IL-1β, IL-1α, IFN-γ IL-6, IL-10, and TNF-α.	[101]
Hemiscorpius lepturus (Experiment with human monocytes and venom-induced human serum)	IL-12, TNF-α.	[108; 109]
Leiurus quinquestriatus Human and animal experimental model (rabbits)	IL-6, IL-8, NO, and TNF-α.	[110; 111; 92]
Tityus serrulatus Human and animal experimental model (rabbits)	IL-1β, IL-6, IL-8, IL-10, IFN-γ, IL-1α, -1β, NO, TNF-α, and GM-CSF.	[105; 112; 103; 100; 104; 113]

2.2.4 Erectile dysfunction by scorpion stings

Particularly in the past 20 years, the venoms of some arthropods have been linked to the mechanism of erectile dysfunction (ED). A persistent inability to maintain or obtain a penile erection that is sufficient for adequate sexual performance is known as ED [114]. The majority of the toxins found in arthropod venoms are ion channel-active, ie, they might elicit physiological alterations in cells directly or indirectly. Neurotransmitter release or inhibition, as well as enzyme activation, may be examples of such alterations. It has been asserted that certain arthropod toxins facilitate cavernosal relaxation and enhance erectile function. As a result, the activation of these toxins in corpus cavernosum (CC) results in NO release has demonstrated by many authors [115; 116; 117; 118].

All members of the Buthidae family of scorpions, except for the hemiscorpion [119], may sting, resulting in priapism, especially in children [120]. The venom of the scorpions *Buthus martensi Karsh* and the African scorpion *Leiurus quinquestriatus quinquestriatus* relaxed the isolated anococcygeus muscle in rats by releasing NO [121; 122]. Toxins isolated from the venom of the scorpion *T. serrulatus*, however, have only been studied as a pharmacological tool in the research of penile erection. According to Gomez et al. (1973) [123], this venom is known to operate on nerve terminals to stimulate the secretion of neurotransmitters like acetylcholine, which activates eNOS in endothelial cells. According to the study by Teixeira and his team [116; 117], the toxin Ts3 isolated from this species causes human corpus cavernosum (CC) relaxation like that elicited by acetylcholine activation. Ts3 delays the kinetics of inactivation [124] via binding to site 3 of Na^+ channels [125].

2.3 Epidemiological study of scorpion stings

Scorpionism is significant in seven select regions of the world on various levels. More than 1.2 million scorpion stings and 3250 deaths caused by scorpion envenoming are registered annually worldwide, and about 2.3 billion people live in areas of scorpionism risk [126; 127; 128; 129]. However, the epidemiological statistics on scorpionism are still limited due to unreported instances and a lack of studies on this issue, despite its widespread prevalence and risk [130; 1]. The continents which are most susceptible to scorpion envenomation are reported and they are Australia, the Near and Middle-East and Mexico, South Asia, Southern Latin America, Saharan Africa (North), Sahelian Afric, South Africa [131; 129; 132].

2.3.1 Epidemiology of scorpionism in America

In several tropical countries, scorpion sting envenomation is a significant public health issue due to its frequent occurrence and possible severity. In South America, scorpions that are deadly to humans belong predominantly to the genus Tityus.

In Brazil, a country in South America, scorpionism is a neglected public health issue, and around 160 species of the scorpion genus Tityus are responsible for stings that are significant from a medical perspective. Four of these Tityus species—*T. serrulatus*, *T. bahiensis*, *T. stigmurus*, and *T. obscurus*—are of medicinal interest [133] and among them; the *T. serrulatus* is the most significant [134; 135; 136]. The number of confirmed deaths from scorpion stings has increased in the previous eleven years, from 61 in 2007

to 90 in 2017, according to the nation's public health system. During this time, there has also been an increase in scorpion sting cases, from 37,370 to 124,982. About 83% of deaths in the past five years (2013 to 2017) happened within 48 hours of being stung [136]. The fatality rate is often less than 0.09% in the other age groups, although it is 0.32% and 0.13%, respectively, for victims under 10 and over 75. However, the fatality rate for children between the ages of 1 and 5 is 0.40 % [136].

In some parts of Brazil, Mexico, and North Africa, this condition is prevalent [137; 138; 139]. The majority of serious illnesses and fatalities are caused by the sting of *T. serrulatus* [134; 135]. Although its origin is uncertain, previous documents point to the Brazilian state of Minas Gerais. Species identification, whether by capture or photography, is crucial, especially if it's one of the four Tityus species with significant medical value. *T. obscurus* needs to be recognized in the Amazon region since it has the potential to induce acute cerebellar impairment [134; 135; 140].

2.3.2 Epidemiology of scorpionism in Asia

Worldwide, the prevalence of scorpion stings is high in some countries of South Asia such as India, Iran, Pakistan, Sri Lanka, Saudia Arabia, Turkey, Spain, France, China, and Mongolia etc [128; 132]. Most of them are associated with the Iranian provinces of Khuzestan, Bueyerahmad, and Kohgiluyeh [141; 132].

Scientists from all over the world have long been interested in the scorpion fauna in Iran from the perspectives of systematics, biology, and ecology. Evaluation of species distribution data is based on research published in the scientific literature until 2012. Scorpion stings were observed all over Iran, and among the 51 species of scorpions found in different parts of the country, the Buthidae family comprises the majority of the scorpion fauna in Iran, accounting for 88.5% of all species and 82% of all genera [131]. The Androctonus genera contain the majority of the recognised medically relevant species within the Buthidae family. Other families of scorpions which are distributed in Iran are Hemiscorpiidae and Scorpionidae. Among provinces of Iran, Khuzestan province stands out for having scorpions and scorpion stings [142]. With 19 kinds of scorpions, Khuzestan is one of the most hazardous regions in the south-west of Iran for scorpion stings. There have been reports on the medical significance, epidemiology, and geographic distribution of scorpions in Iran [143; 131; 144].

Due to the importance of scorpion stings and the lack of epidemiological information on this concern in public health, the study was conducted to gather fresh information on scorpion stings in Iran. There are different types of weather in Iran, including a hot summer and a cold, snowy winter. One study reported that between 2002 and 2011, there were 54.8 to 66 scorpion stings per 100,000 people. These differences were likely caused mostly by diverse climatologic factors and preventative strategies. Around 3250 deaths are predicted to occur worldwide each year, affecting 1.2 million individuals. According to Chippaux and Goyffon (2008), the global mean rate of sting occurrences per 100,000 people per year is approximately 17.14 [1]. It demonstrates that Iran experiences higher scorpion stings than the global average. The crucial fact is that, from 2002 to 2011, scorpion stings occurred at a similar incidence of 54.8 to 66 per year. According to research conducted in Kashan, central Iran [145], and Ahvaz, south-west Iran, scorpion stings were most common among people aged 15 to 34 [146]. The highest incidence of scorpion sting cases in 2011 took place in the summer (44.16%). This is consistent with findings from Iran [147], Saudi Arabia [18], Turkey [148], and Turkey [145; 131; 146; 149]. According to their data, 49.7-93.4% of scorpion sting incidents happened in the summer. The severity of envenoming depends on the variability of scorpion venoms. Therefore, identifying the species that caused the sting is crucial and may have an impact on the clinical procedures used to treat the patient.

Scorpion stings cases can also be found in Saudi Arabia, Turkey, Spain, France, China, and Mongolia due to their climate and geographic location. Recently, two studies on scorpion stings were reported in Pakistan, one in the Sargodha district of Punjab [150] and the other in the Lasbella district of Balochistan [151].

In India, the western states of Maharashtra, Saurashtra, Kerala, Andhra Pradesh, Tamil Nadu, and Karnataka are regularly affected by morbidity and mortality brought on by scorpion stings. According to a case study involving 141 children who were admitted to the Government Raja Mirasdhar Hospital in Thanjavur, southern India, after being stung by a *M. tamulus* species, children between the ages of 1-3 and 7–12 showed the most adverse effects to envenomation. Eight individuals had priapism, and five of them were older than six years. The fatal and life-threatening sting effect of pulmonary oedema was observed in one patient older than 6 years [152]. According to records from a tertiary care and teaching hospital in southern India, 50 patients who were stung by a *M. tamulus*

species, had dyspnea (13, 26%), chest discomfort (9, 18%), vomiting (6, 12%), sweating (5, 10%), nausea (3, 6%), priapism (7, 14%), and piloerection (6, 12%) [153].

An epidemiological study conducted in Mahad (200 km south of Mumbai, Western India) from 1984 to 1995 also showed that children <16 years tend to respond more rapidly to MT stings [154]; out of 293 patients, six deaths were reported before hospital arrival. Patients were further divided into three broad groups based on the clinical symptoms; i) 111 (38%) patients exhibited hypertension within 1-10 h (mean 3.5 h), ii) 87 (30%) patients with tachycardia reported within 1-24 h (mean 6.7 h), and iii) 78 (24.5%) patients with pulmonary oedema reported within 6-24 h (mean 8 h) post scorpion sting [155; 154].

Twenty-three MT stings have been documented in three localities of Jaffna, Sri Lanka, consisting of 13 (57%) males and 10 (43%) females where the average age was 30, while 5 (22%) of the cases were children under the age of 12. All patients had signs of envenoming, either local or systemic, upon admission to the hospital [156; 157]. While the coastline regions and the nearby islands have limited vegetation on sandy soil, the central region of the district is rich with overgrazed red soil. Over a year, there were 90 admissions to hospitals with a history of scorpion stings. Of those, the *M. tamulus* was the offending scorpion in 84 of the cases, and black scorpions primarily stung the others. The offending scorpions were identifiable in 23 MT sting cases (confirmed cases), and in the remaining 61 cases (n), the victims or witnesses had sightings of the offending scorpions but were unable to capture them [156].

From 2009 to 2014, 33 reports of scorpion stings were made at the Rims Teaching Hospital in Raichur, Karnataka, India, of which 22 were caused by the Indian black scorpion and 11 by the Indian red scorpion. The patients experienced hypotension, hypertension, cutaneous symptoms, bradycardia, and drowsiness [158]. It has come to light that scorpion stings in the northern region of Sri Lanka, particularly in the Jaffna District, were causing severe envenoming.

2.3.3 Epidemiology of scorpionism in Africa

Despite the diversity of scorpion species found in South Africa, statistical data on the frequency and severity of scorpion envenomation is limited. A study conducted with 52,163 consultations in Tygerberg Poisons Information Centre (TPIC) reported 740 (1.4%) cases involved in scorpion stings. 146 (19.7%) of them were considered to be

significant envenomations. Adults (>20 years) accounted for 71.4% of the victims in these cases, and they were more likely to suffer from less harmful stings (OR 0.57; 95% CI 0.37 to 0.86). In 356 (48.1%) cases with significant associations to decreased severity (OR 3.51; 95% CI 1.9 to 6.3), the TPIC was contacted within six hours of the sting occurrence. However, only 15% of the scorpions were able to be identified [159].

2.3.4 Epidemiology in Mexico

The harmful scorpion *Centruroides infamatus infamatus*, which lives in the Mexican state of Guanajuato, has been seen to become more active during the warmer months [160]. Similar results were seen in Argentina, where scorpion sting incidence increased from October to April due to *Tityus trivittatus* [161]. Dehesa-Dávila correlates a decline in scorpion stings with the arrival of the rainy season [162; 163]. The majority of scorpions are nocturnal that spend the daytime in burrows, rocks, or leaf litter. However, Chowell et al [162] report a significant and positive correlation between Mexico's minimum temperature and scorpion activity. Instances of scorpion stings increased to their maximum levels in 2000 and 2001 when the lowest temperature fell to 19.4°C and 18.8°C, respectively. This association is consistent with studies from Brazil and Argentina [161; 160]. The authors noticed a "threshold" relationship between scorpion sting frequency and pluvial precipitation [162]. There were extremely few scorpion stings when rainfall was lower than 30 mm/month; when rainfall was greater than 30 mm/month, scorpion sting incidence was independent to actual rainfall. This might be as a consequence of rain, which disturbs scorpions and forces them to seek for new hiding places.

2.4 Limitation of antivenom therapy and their improvement protocol for better treatment of scorpion stings

Antitoxins and antivenoms have been used successfully for more than a century. Throughout this period, these products have consistently demonstrated their ability to cure infections and envenomation in a very successful manner. For each antivenom, the indications, route of administration, and frequency of adverse effects vary substantially. Since the earliest antivenom was produced at the start of the 20th century, the technique to produce antivenom has gradually changed. Early antisera were frequently associated with severe reactions including serum sickness. In theory, new $F(ab')_2$ products could theoretically result in less immunological responses when used in clinical applications

since they are produced using pepsin digestion together with precipitation of undesired protein and albumin serum fractions. The risk of morbidity or fatality from antivenom itself varies greatly, which has significant effects on public health [164; 165]. More specifically, less refined products may have a high enough risk of anaphylaxis to prevent such usage, thereby limiting access to vital emergency therapy. More refined products may be safe enough for everyday use in geographically inaccessible clinics.

According to a study, antivenom was administered to 1534 individuals with scorpion envenomation in Arizona and Mexico, ranging in age from 0.1 to 90.5 years. After treatment with antivenom, three patients (0.2%) experienced acute antivenom infusion responses, including urticaria, urticaria with dyspnea, and panic attack. No one got the entire symptoms of serum sickness, although eight (0.5%) of the participants exhibited rashes that were indicative of Type 3 immunological responses. Two pregnant women had envenomation treatment in the first trimester; one of them later had a spontaneous abortion. Thus before using antivenom for therapeutic purposes, these procedures can be used to assess the quality, effectiveness, stability, and safety of the antivenom [166; 167]. There is a high demand to discover alternative approaches for better improvement of scorpion stings treatment devoid of any adverse effects.

Furthermore, the failure of ASAs to immunorecognized the venom toxins due to the presence of a low proportion of venom-specific antibodies in ASA is another hurdle for efficient hospital management of scorpion sting victims [168; 169]. Commercial ASA and prazosin (an α1-adrenergic inhibitor), frequently in conjunction with insulin, are used in clinical treatment to reduce several complications caused by scorpion venom. These therapies do have certain limits, though, which renders it necessitate to investigate ethno-medicines, particularly traditional medicinal plants, to cure scorpion stings. A list of more than 200 medicinal herbs that have been utilised for treating scorpion stings traditionally in various nations. Although various myth-based treatments for scorpion stings are used, there is no actual data to support this aspect of conventional knowledge [170]. To establish their neutralisation potency against scorpion stings, only 38 traditional medicinal plant extracts have been examined *in-vivo* and *in-vitro*. Even though a small number of bioactive plant components with scorpion venom neutralisation potency have been identified, they are not currently commercially available for use in clinical settings [170]. Therefore, such drawbacks of conventionally used antivenom therapy demand

modern science to develop an effective, specific, alternative, and advanced treatment for scorpion envenomation.

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