ISSN 2449-8955

European Journal of Biological Research

Review Article

Scorpion venom: pharmacological analysis and its applications

Mukesh Kumar Chaubey

Department of Zoology, Mahatma Gandhi Post Graduate College, Gorakhpur - 273001, U.P., India;
E-mail: mgpgc@rediffmail.com

Received: 14 July 2017; **Revised submission:** 25 September 2017; **Accepted:** 27 September 2017

DOI: http://dx.doi.org/10.5281/zenodo.998076

ABSTRACT

Scorpions belong to class: Arachnida, order: Scorpionida represented now by approximately 1500 species. These are one of the most ancient group of the animals on the earth conserving their morphology almost unaltered and are the most successful inhabitants of the earth. Scorpions when stimulated secrete venom which is a cocktail of variable concentration of neurotoxins, cardiotoxins, nephrotoxins, hemolytic toxins, phosphodiesterases, phospholipases, hyaluronidase, glucosaminoglycans, histamine, seratonin, tryptophan and cytokine releasers. According to an estimate, frequency of deaths caused by scorpion sting is higher in comparison to that of caused by snake-bite. Almost all of these lethal scorpions except *Hemiscorpious* species belong to scorpion family Buthidae comprising 500 species. Scorpion venoms show variable reactions in envenomated patients. However, closer the phylogenic relationship among the scorpions, more similar the immunological properties. Furthermore, various constituents of venom may act directly or indirectly and individually or synergistically to exert their effects. Scorpion stings cause a wide range of conditions from severe local skin reactions to neurologic, respiratory and cardiovascular collapse. Lethal members of Buthidae family include *Buthus*, *Parabuthus*, *Mesobuthus*, *Tityus*, *Leiurus*, *Androctonus* and *Centruroides*. Besides their lethal properties, scorpion venoms have some unique properties beneficial to mankind. These contain anti-insect, antimicrobial and anticancer properties and thus, can play a key role in the insect pest management programmes, treatment of microbial infection and in the treatment of various cancer types.

Keywords: Scorpion venom; Envenomation; Neurotoxins; Ion channel blockers; Anticancer peptide; Antivenom.

1. INTRODUCTION

Scorpion sting is a major health problem in under developed tropical countries especially in poor communities. According to an estimate, frequency of deaths caused by scorpion sting is higher in comparison to that of caused by snake-bite [1]. Scorpions belong to class: Arachnida, order: Scorpionida. Scorpion has flattened and elongated body with four pairs of legs, a pair of claws and a segmental tail that has a poisonous spike at the end. Scorpion varies in size according to age and species from 1-20 cm in length. These can be found outside their normal territory when they accidentally crawl into luggage, boxes, containers or shoes, and are transported to home via human unwillingly. These are one of the most ancient group of the animals on

the earth represented now by approximately 1500 species conserving their morphology almost unaltered [2, 3].

Scorpions are not aggressive and do not hunt but wait for its prey. Scorpions being nocturnal in habit and capture its prey during night. They hide in crevices and burrow during day time to avoid the light. Human stinging occurs accidentally when scorpions are touched during resting and most of the stings occur on hands and feets. Scorpions are well equipped with a pair of pincer like pedipalps. Thus, peoples question why they need to produce venom at the same time. This is because most scorpions are opportunistic predators lacking the speed of its prey like insects and thus they are not choosy in their prey selection. Also obtaining relatively large prey like mouse and large beetles is a quite tough task for a scorpion. In such condition, pedipalps may prove insufficient to manage prey as quickly as possible. Thus, venom which is nature's gift provided to scorpions comes into play. A second advantage of venom is the presence of enzymes as venom's constituents with diverse activity. These enzymes initiate the process of digestion in tissue of the prey stung before consumption. Scorpion venom is an effective defensive device, which serves to deter and incapacitate the opponent. Hissing and aggressive defense posture is usually enough to deter most animals including human. When deterrence proves inadequate scorpions defend itself by injecting venom into the body of enemy. Thus, the main purpose of production of venom in scorpions is to secure food and self-protection.

Venom glands, the factory of scorpion's venom are located on the lateral side of tip of sting. These are made of different types of tall columnar cells. Of these cells, one type produces toxins while others produce mucus. Potency of scorpion's venom varies from species to species with some producing only a mild flu while other producing death. Scorpion stings cause a wide range of conditions from severe local skin reactions to neurologic, respiratory and cardiovascular collapse. Scorpion venoms exert their action mainly by affecting specific functions of the ion channels [4-6]. Among well-characterized toxins peptides from venom of the scorpion, most of them belong to family Buthidae. Buthoid venom has been reported for its severe consequences against a wide variety of vertebrate and invertebrate organisms and its toxicity is attributed to the presence of a large variety of basic polypeptides having three to four disulfide bridges [4, 7]. Due to heterogeneous nature, scorpion venoms show variable reactions in envenomated patients. However, closer the phylogenic relationship among the scorpions, more similar the immunological properties. Furthermore, various constituents of venom may act directly or indirectly and individually or synergistically to exert their effects. In addition, differences in amino acid sequences of each toxin accounts for their differences in function, pharmacology and immunology. Thus, any alteration in amino acid sequence may result in modification of function, pharmacology and immunology of toxin.

Differences in pathogenecity and level of toxicity of scorpion venom are actually due to diversity in toxin peptides and differences in amino acids in active site region of toxin peptides. This leads to diversification in their mode of action in different venomous scorpion groups in different climatic conditions and finally results into ecological adaptation in due course of evolutionary journey. Scorpions when stimulated secrete a small quantity of transparent venom called prevenom. If stimulation continues, cloudy, dense and white coloured venom is released subsequently. Prevenom contains a concentration of high K^+ salt and several peptides including some that block K^+ channels. This prevenom causes significant toxicity and scorpions use it as a highly efficacious predator deterrent and for immobilizing small prey while conserving metabolically expensive venom until a certain level of stimuli is reached [8]. That is why scorpions are known to be economical in their use of venom.

Production and storage of venom is an expensive metabolic process especially for species of extreme ecosystems. Other than antimicrobial peptides, all neurotoxins in venom are highly folded disulfide bridged molecules [9]. Low yields and reduced expression of these highly folded peptides in recombinant system indicates unique and difficult folding and storage requirement [10]. About fifty scorpion species distributed throughout the world have been proved lethal to human [11, 12]. Almost all of these lethal scorpions except *Hemiscorpius* species belong to scorpion family

Buthidae comprising 500 species. Lethal members of Buthidae family include *Buthus*, *Parabuthus*, *Mesobuthus*, *Tityus*, *Leiurus*, *Androctonus* and *Centruroides*. Common scorpions and their distributions are:

1. *Buthus*: Mediterranean area
2. *Parabuthus*: Southern and Western Africa
3. *Mesobuthus*: Asia
4. *Tityus*: Central and South America
5. *Leiurus*: Northern Africa
6. *Androctonus*: Northern Africa to South-East Asia
7. *Cetruroides*: South-West USA, Mexico, Central America
8. *Heterometrus*: Asia
9. *Pandinus*: Tropical Africa and Arabian Peninsula.

Scorpions mostly occur in temperate and tropical habitats of the world. They are well adapted to survive in extreme thermal environments, sometimes constituting a major portion of the total animal biomass in such environments. These are considered among the most successful inhabitants of the earth [13, 14]. Although numerous factors contribute to the success of scorpions, the ability to produce and deliver highly toxic venom is an important determinant of their success.

Scorpion venom is composed of water, salts, biogenic amines, peptides and enzymes. Venom of several scorpion species has been well characterized and various toxin peptides possessing the majority of biological activities have been isolated [15]. In venom mixture, there are many peptides that are specifically active against vertebrates, invertebrates or both. Toxin peptides of all these three groups are well characterized and includes peptides that target all the major ion channels such as Na^+, K^+, Cl^-, Ca^{++} and rynodine sensitive Ca^{++} channels [15, 16]. Potency of venom is mainly due to its ability to target multiple types of ion channels simultaneously resulting in a massive and recurring depolarization of nerve fibres that disables or kills prey or predators.

Generally scorpion venom possesses variable concentration of neurotoxins, cardiotoxins, nephrotoxins, hemolytic toxins, phosphodiesterases, phospholipases, hyaluronidase, glucosaminoglycans, histamine, seratonin, tryptophan and cytokine releasers. The most potent toxin is neurotoxin, which is divided in two classes viz. short chain and long chain peptides. Toxin peptides of both these classes are heat stable with low molecular weight. These are responsible for cell impairment in nerves, muscles and the heart by altering ion channel permeability. Long chain polypeptide neurotoxins cause stabilization of voltage dependent Na^+ channel in open position leading to continuous prolonged repetitive firing of somatic, sympathetic and parasympathetic neurons. These repetitive firings result in autonomic and neuromuscular over excitation preventing normal nerve impulse transmission. Further, it results in excessive release of neurotransmitters such as acetylcholine, glutamate, aspartate, epinephrine and norepinephrine.

Short chain polypeptide scorpion toxins are K^+ channel blockers. Binding of these toxin peptides is reversible but with different binding affinities. Stability of these neurotoxins is due to four-disulfide bridges that fold neurotoxin into a very compact three-dimensional structure, thus making it resistant to variation in hydrogen ion concentration and temperature. However, reagent that can break disulfide bridges can inactivate this toxin by unfolding it. Antigenicity of these toxins depends on the length and number of exposed regions out of the three-dimensional structure.

Fat tailed scorpion, *A. australis* has many toxin peptides, which are selectively lethal to mammals. This selectivity of venom can hardly be explained by food choice. This suggests a possible selective pressure for venom production against mammalian predators. It also helps to acquire other vertebrate prey as well. Also, if food acquisition is the main selective pressure for venom against vertebrates, then there should be higher composition of vertebrate toxins in large species like *P. imperator*. The hypothesis for deterrence is supported by composition of venom. Besides, other pathological and physiological effect, serotoxin, which is a constituent of scorpion venom, also causes pain similar to that caused by apamin of honey bee. In fact, immediate stimulation of pain is one of the most important properties of scorpion venom. Generally, toxin factors that initiate pain do not cause death. This certainly is the result of other components present in the cocktail of substances in venom. Now, it is a well-known fact that among all different scorpion toxins, neurotoxins are the most lethal peptides that cause high mortality in animals. Scorpions use their pincers to grasp their prey and

then arch their tail over their body to inject their venom into the prey, sometimes more than once. Scorpions regulate how much venom should be injected with each sting. The striated muscles in the sting regulates amount of venom ejected, which is usually 0.1-0.6 mg. If entire supply of venom is used, scorpion must require several days to regain venom supply.

Although poisonous scorpions are classified taxonomically into several genera, yet the mode of action of their venom is quite similar. Scorpion venoms contain neurotoxic peptides in low abundance with great diversity in their mode of action. These neurotoxin peptides are low in abundance in a complex mixture of venom having a majority of the biological effects towards the affected victim. Stings affect peripheral nervous system resulting in symptoms like intense pain at the site of sting, altered heart activity and paraesthesia [17].

In an experiment with labeled scorpion venom, amount of venom venom was found 28% in blood, 30% in muscle, 13% in bone, 12% in kidney and 11% in liver within five minutes after intravenous administration. Scorpion venom is excreted through renal and hepaticbiliary pathways. The maximum renal uptake of 32% at thirty minutes drops to 22% at three hours suggesting that excretion of venom through kidney is slow [18]. Scorpion venom in the animal body has a half-life of 24 hour indicating a slow clearance with mean residence time of 33.7 hours in the body and 26 hours in the peripheral compartments [19].

2. INSECTICIDAL PROPERTY OF SCORPION VENOM

Due to species-specific activity of scorpion toxins, efforts have been made to identify insect selective toxins that can be used to develop biopesticides as a safer alternative to replace chemical insecticides [20-22]. On the basis of mode of action, anti-insect scorpion toxins have been divided into three classes viz. (i) alpha toxins which are strictly selective for insects (ii) excitatory insect selective scorpion toxins, and (iii) depressant insect selective neurotoxins [23-25]. Anti-insect α-toxin peptides bind to voltage-dependent sodium channels with high affinity [26].

Excitatory toxin causes a repetitive firing of axon accompanied by a small depolarization [27]. On the other hand, depressant toxin produces an inhibition of excitability due to depolarization of axon. Depressant toxins cause a decrease in sodium peak current and induce a constant inward current at negative membrane potential [28]. These effects are similar to that of the beta toxins active against vertebrate systems [29]. Several insect selective toxins have been identified from scorpion venom of different geographical regions [16, 17, 30, 31]. AalT, a single chain neuropeptide isolated from *Androctonus australis*, has been proved insectotoxin by causing fast excitatory paralysis by presynaptic effect on insects's motor nerve resulting in a massive and uncoordinated stimulation of skeletal muscles. The neuronal repetitive activity is attributed to an exclusive and specific perturbation of sodium conductance as a consequence of toxin binding to external loop of insect's voltage dependent Na^+ channel and modification of its gating mechanism [32]. Three toxin peptides (AaHIT1, AaHIT2, and AaHIT3) have been isolated from *Androctonus australis* venom which act against insect and are used as potential insecticidal agents. AaHIT1 gene linked to a sendai virus has been transformed to mosquitoes by viral infections, which upon transformation express lethal toxins/ proteins and resulted in death of host [33]. The other two toxin peptides are also insect specific similar to AaHIT1 [39].

An anti-insect toxin peptide Lqh alpha IT has been isolated from *L. quinquestriatus* venom which causes a unique mode of paralysis in blowfly larvae [35]. Like excitatory and depressant insect toxins, Lqh alpha IT is highly toxic to insects but it differs from these in two important characteristics: (a) Lqh alpha IT lacks a strict selectivity for insects, highly toxic to crustaceans and also low toxic to mice. (b) It does not displace an excitatory toxin AalT from its binding site in insect neuronal membrane, which confirms that the binding site for the Lqh alpha IT is different from those imparted by excitatory and depressant toxins. Bot XIV isolated from *B. occitanus occitanus* is also an insecticidal toxin peptide but it does not show toxicity against mammals. This toxin peptide is highly antigenic in mice with the resulting antibodies having significant effectiveness in neutralizing other more toxic

proteins. Another antiinsect toxin Lqh III isolated from *L. quenquestriatus* affects sodium current in cockroach giant axon and prolongs action potential [36]. From *M. tamulus*, a short toxin peptide has been characterized which shows toxic effects against *Helicoverpa armigera* [37]. Gawade [38] has reported anti-insect toxin peptides, C56, from *Buthus* that has been shown to induce Ca^{++} dependent spontaneous excitatory activity in *Drosophila* larvae. Anti-insect toxin peptides characterized can be used in constructing genes and their *in vitro* expression product can be used as a replacement for synthetic pesticides. Albert et al. have expressed a synthetic gene encoding insecticidal neurotoxin of *A. australis* (AaIT) in NIH/3T3 mouse fibroblast cells under transcriptional control of a murine retro-viral long terminal repeat. Toxin peptides secreted in culture medium has been found toxic against yellow fever mosquito larvae but with no toxic effect on mice [20]. Genes of scorpion anti-insect toxin peptides mainly neurotoxin peptides have been used with recombinant baculovirus. These genes have been selected to avoid human and other non-target neurotoxicity as much as possible. In such aim of insect control, depressant toxin has been found more effective than excitatory toxin in recombinant baculovirus [39].

From a strict agro-technical point of view, two main points should be considered regarding the involvement of toxin peptide genes in plant protection (i) these act as a factor for genetic engineering of insect infective baculoviruses resulting in potent and selective bioinsecticides, and (ii) these must show pharmacological flexibility as a device for insecticide resistance management [32]. Indian red scorpion *M. tamulus*, known for its severe toxicity [40, 41], received little attention in this regard and only few toxin peptides have been reported for their insecticidal properties [42], while no such toxin peptides active against insects have been characterized from *Heterometrus* species till now.

3. ANTIMICROBIAL PROPERTY OF SCORPION VENOM

Since the discovery of antimicrobial peptides in invertebrates [43], more than hundreds of antimicrobial proteins have been characterized in both invertebrates [44] and vertebrates [45] with a wide phylogenic distribution including humans [46]. These have been reported in skin, epithelial cells and blood of vertebrates as well as in insect haemolymph and venomous secretions of bees, hornet, spider and scorpions [47-49]. These toxins are small basic peptides with variable length, structure and sequence. These antimicrobial peptides appear to form channels or pores in cell membrane inducing cell permeation and break down of cellular physiology [50]. These antimicrobial peptides have broad-spectrum, nonspecific activity against a wide range of microorganisms including viruses, gram-negative and gram-positive bacteria, protozoa, yeast and fungi, and may also be hemolytic and cytotoxic to cancerous cells [51, 52]. Antimicrobial peptides from scorpion venom are short peptides consisting of 10 to 50 amino acid residues with a net positive charge ranging from +2 to +9 and the proportion of hydrophobic residues are equal or more than 30% of total amino acids residues [53]. The positive amino acid residues are separated by patches of hydrophobic amino acids [54]. These antimicrobial peptides are usually cationic, amphipathic, α-helical peptides of low molecular eight (2-5 kD). Some peptides, such as hadrurin, are highly potent against both Gram-positive bacteria and Gram-negative bacteria without preference, while others show selective activity against either Gram-negative bacteria (parabutoporin) or Gram-positive bacteria (IsCT and BmKn2) [55, 56].

The pore forming antibacterial peptides of scorpion venom can be divided into two groups, depending on their primary and secondary structures: (a) linear, alpha helical peptides without cysteine residues, and (b) cysteine rich peptides that form a beta sheet or beta sheet and alpha helical structures [57]. Besides acting by destabilizing membrane structure and changing ion permeabilities, pore forming peptides can influence cell functioning by interacting with intracellular signaling molecules such as G-proteins [58]. Although many antimicrobial peptides have been described in insects [59], several antimicrobial peptides have been isolated and characterized from scorpions including several cysteine-containing peptides from haemolymph of scorpion *L. quinquestriatus hebraeus* [60] and *A. australis* [61].

The earliest peptide toxin ever studied was androctonin isolated from *A. australis* venom [62]. Androtonin shows potent antibacterial activity against both Gram-positive and Gram-negative bacteria [62]. Moerman et al. have also reported antifungal activity of androtonin [63]. Powers and Hancock have reported the antibacterial activity of parbutoporin (from *P. schlechteri*) and opistoporins (from *Opistophthalmus carinatus*). These peptide toxins target G-proteins for membrane lytic activity [64]. VpAmp1.0 and VpAmp2.0 peptides isolated from *Vaejovis punctatus* inhibit growth of both Gram-positive (*Staphylococcus aureus* and *Streptococcus agalactiae*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, yeasts (*Candida albicans* and *Candida glabrata*) and two strains of *Mycobacterium tuberculosis* [65]. Opisin peptide isolated from *Opistophthalmus globrifrons* is a cationic, amphipathic, and α-helical molecule with 19 amino acid residues without disulfide bridges. This peptide inhibits growth of the some Gram-positive bacteria [66]. Stigmurin isolated from Brazilian yellow scorpion *Tityus stigmurus* venom gland shows antibacterial and antifungal activity [67]. Ctriporin isolated from *Chaerilus tricostatus* shows a broad-spectrum of antimicrobial activity and is able to inhibit antibiotic resistant pathogens, *Staphylococcus aureus* strains [68]. Hp1404 isolated from *Heterometrus petersii* is an amphipathic α-helical peptide with inhibitory activity against gram-positive bacteria like *Staphylococcus aureus* [69].

Buthinin, a three disulfide bridged bactericidal and fungicidal peptide, androctonin, with two disulfide bridges from *A. australis* venom and scorpine, a 75 residue antimicrobial peptide from *P. imperator* venom have been characterized [61, 70]. Alpha-helical proteins containing antimicrobial properties have been reported from *Hadrurus aztecus* venom [71] and *P. schlechteri* [72]. Pandinin 1 and pandinin 2 with antimicrobial property have been isolated from *P. impretor* venom [9]. Most of these antimicrobial peptides share some common characteristics such as their low molecular mass, presence of multiple lysine and arginine residues and their amphipathic nature. Their site of action is cytoplasmic membrane where they destabilize its lipid package and produce transient channels and disturb ion permeability across the membrane [73, 74].

Heterometrus xanthopus venom contains antimicrobial peptides like hadrurin, scorpine, Pandinin 1 and Pandinin 2. These peptides are able to kill antibiotic-resistant strains of *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 14506. Two antimicrobial peptides have been identified from the venom of North African scorpion, *A. aeneas*. These peptides show antimicrobial activity against the Gram-positive bacterium, *S. aureus* and the yeast, *C. albicans*, but do not affect Gram-negative bacterium, *E. coli* [75]. Scorpion *Leiurus quinquestriatus* venom shows significant broad-spectrum antimicrobial activity against *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans* and *Candida glabrata* [76]. Despite of their high minimum inhibitory concentration in comparison to other antibiotics, their broad spectrum of activity and speed of action makes them good candidates for drug delivery and for a number of other possible applications in pharmacological research [52].

4. ANTICANCER PROPERTY OF SCORPION VENOM

Cancer is a major health problem all over the world [77]. Treatment of cancer involves different clinical approaches including surgery, chemotherapy, radiotherapy, gene therapy, hormone therapy and immunological therapy either alone or in combinations. All of these approaches have its own advantages and disadvantages and mainly depend on the type and stage of cancer. Recent advancement in cancer therapy includes synthesis of peptides and proteins through DNA technology and production of monoclonal antibodies specific for oncoproteins. After realizing the medicinal use of anticancer proteins and peptides, many proteins of animal origin have been isolated. De Carvalho et al. isolated and characterized lectins (polyvalent carbohydrate binding proteins of non-immune origin) from snake *Bothropos jararacussu* venom which serve as an interesting tool by inhibiting tumor cells of human breast and ovarian cancer [78]. Some other group of scientists reported

anticancer effect of other lectins and toxins from some other snake venoms also [79-81].

Scorpion venom contains a number of polypeptides with diverse biological activities. It has been earlier reported that venom of some scorpions has high histopathological and necrotic effects in human and animals [82, 83]. However, for the first time, Omran has reported anticancer property of *Leiurus quinquestriatus* venom on human breast cancer cell lines [84]. According to Bruses et al. [85] some toxins bind to a specific receptor in the membrane before they can exert their action. Anticancer effects of scorpion venoms have been evaluated in various types of cancers as glioma, neuroblastoma, leukemia, lymphoma, breast, lung, prostate and pancreatic cancer. These venoms produce anticancer effects by blocking specific ion channels, inhibiting invasion and metastasis of cancer cells and activating intracellular pathways leading to cell cycle arrest and apoptosis [86-88]. Venoms from various scorpions have been reported to prevent propagation of different cell lines such as prostate cancer, human leukemia and neuroblastoma [89-91]. Venom of *A. crassicauda* inhibited proliferation of human neuroblastoma cell lines through arresting S-phase and induction of apoptosis [92]. Almaaytah et al. characterized the cytolytic peptides AamAP1 and AamAP2 from the venom of North African scorpion *A. amoreuxi* [93]. They reported that the natural peptides AamAP1 and AamAP2 show moderate antiproliferative activity against LNCaP, U251, PC3 and HMEC-1 cell lines. The venom peptide Acra3 from *A. crassicauda* induces cytotoxic effect on mouse brain tumor cells (BC3H1) through both necrotic and apoptotic pathways [90]. Venom from the Buthidae scorpions *A. bicolor, A. crassicauda* and *L. quinquestriatus* show strong anticancer activity on colorectal and breast cancer cell lines through decreasing cell motility and colony formation of cancer cells [89].

5. ION CHANNEL BLOCKING PROPERTY OF SCORPION VENOM

Scorpion venom contains several small neurotoxic peptides, which selectively act on various types of ion channels. These toxin peptides have been extensively used as valuable biochemical and pharmacological tools to characterize and discriminate various ion channel types that differ in ionic selectivity, structure and function. These neurotoxins affect victim by interfering with ionic balance and ion channel activity in excitable cells. Binding of scorpion toxins to target ion channels is known to occur through multiple interactions [94]. Numerous amino acid residues that determine the binding property to target ion channels have been characterized [15]. In addition, α-scorpion toxins are known to inhibit or slow down the Na^+ ion channel. Scorpion toxin peptides can be divided into four groups on the basis of their target ion channels. The first class belongs to toxin peptides acting on the Na^+ channel, which consist of 60-70 amino acid residues and four intermolecular disulfide bonds. These long chain toxin peptides modulate activation or inactivation of Na^+ channels [15]. These toxin peptides alter kinetics of Na^+ channel opening and closing in excitable cells [95]. Scorpion toxins affecting voltage-gated Na^+ channels have been divided into two groups α- and β-toxins on the basis of their electrophysiological effects and binding properties. Alpha-toxins bind in a voltage dependent manner and inhibit depolarization of action potential while beta-toxins bind in a voltage independent manner and modulate the activation phase of action potential [96]. Makatoxin 1 and bukatoxin, members of α-scorpion toxin family isolated from *B. martensi* venom show pharmacological action similar to other α-toxins on neuronal voltage sensitive sodium channels [97]. The toxin peptides from Buthidae family prolong Na^+ ion activation phase of action potential while toxins from Centrurinae and Tityinae venom affect Na^+ activation phase [98]. Ts-gamma, a neurotoxin of *T. serrulatus* produces very complex cardiological effects characterized by an initial reduction of both rate and contractile force followed by an increase in force and reduction of rate. This contraction finally reduces due to release of acetylcholine from vagal endings [99]. This toxin apparently produces these effects on cell currents primarily by retarding activation of cardiac sodium channels [100]. Gawade et al. [38] have isolated and characterized a toxin peptide Lqh1β1 from *Leiurus quinquestriatus hebraeus* venom. It competes with anti-insect and anti-mammalian α-toxins for its binding site on Na^+ channel. It also competes with an anti-mammalian α-toxin for its binding site.

The second class of toxins includes K^+ ion channel blockers. These toxin peptides consist of 23-40 amino acid residues having three to four disulfide bonds. Bmpo2, a 28 amino acid residues peptide from *B. mortensi* venom shows very low inhibition of apamine sensitive Ca^{++}-activated K^+ channel [101]. Neurotoxic peptides tamulotoxin (TmTx) and iberiotoxin (IbTx) from *M. tamulus* having 37 amino acid residues and three disulfide bridges have shown to cause Ca^{++}-activated K^+ channel blockage [102]. Other scorpion toxins with shorter polypeptide chain having less than 40 amino acid residues such as charybdotoxin and kaliotoxin also act on this channel [31, 103, 104]. Although numerous known scorpion toxins differ in size, sequence and biological activity, they all share a common structural motif consisting of antiparallel sheet linked to an amphipathic helix and an extended N-terminal fragment by three disulfide bridges [30]. This motif is also present in insect defensins, a family of inducible antibacterial peptides isolated from a variety of insects where they present a key element of the innate host defense against microorganisms [105].

Romi-Lebrun et al. have isolated four peptideyl inhibitors of small conductance Ca^{++}-activated K^+ channels from *B. martensi* [101]. *C. noxius* contains β-toxin which blocks voltage gated K^+ channels by binding to site different than that of other beta toxins [106]. Margatoxin of *C. margaritatus* is a potent K^+ channel blocker selecting for only one sub-type of K^+ channel. This particular K^+ channel is directly involved in lymphocytes activation and blocks lymphocyte activation and production of interleukin-2 by human T-lymphocytes [107-109]. Agitoxin of *Leirus* venom binds to external pore entry pathway of shaker K^+ channel as well as mammalian homologues [110]. This toxin is related K^+ channel neurotoxins but forms a new subclass of scorpion derived K^+ channel inhibitors [100]. Scyllatoxin (laiurotoxin1) *Leiurus* binds to high conductance Ca^{++}-activated K^+ channels [111]. *P. imperator* venom contains peptides that binds and blocks voltage-gated K^+ channels [112, 113]. Two toxin peptides viz. Imperatoxin A and Pil have been identified from *P. imperator* venom. Imperatoxin A selectively activates skeletal-type ryanodine receptor [114] and may prove a useful tool to identify regulatory domains critical for channel gating and to dissect the contribution of skeletal-type Ca^{++} release channel/ryanodine receptor to intracellular Ca^{++} wave forms generated by stimulation of different ryanodine receptor isoforms [115]. Pil toxin peptide selectively blocks shaker K^+ ion channels [116]. Many researchers have isolated short chain polypeptides like Ibtx from *B. tamulus* [117], Titustoxin V from *T. serrulatus* [118], Osk-1 from *Orthochirus scrobiculosus* [119] and Chtx from *B. martensi* [101]. These toxin peptides are potent inhibitors of voltage-gated K^+ channels. Dhawan et al. (2003) have isolated a short toxin peptide BTK-2 from *Buthus tamulus* that inhibits K^+ channel [37]. More et al. have reported a toxin peptide (PGT) fron *Palamneus gravimanus*. This toxin peptide selectively blocks the human cloned voltage-gated K^+ channel [120].

The third class of scorpion toxins acting on Cl^- channels has 35-37 amino acid residues with four disulfide bonds [121]. Chlorotoxin from *L. quinquestriatus* shows highest homology with short insect toxin [122]. The forth class includes toxins acting on Ca^{++} channels. These are short peptides with 25-35 amino acid residues [123, 124]. Kurtatoxin isolated from *P. transvaalicus* venom has been reported to inhibit voltage gated Ca^{++} channel [125].

Less than 1% of the estimated 0.1 million distinct peptides expected to exist in scorpion venom are known. It can be speculated that natural selection co-evolved distinct types and subtypes of receptors of ion channels in various groups of animals. At the same time scorpion evolved specific toxins designed to interfere with normal function of ion channels and to provide one way for scorpions to capture their prey or defend themselves from predators.

6. CARDIOTOXIC PROPERTY OF SCORPION VENOM

Scorpion venom induces complex cardiac disorders in several animal species [126-128]. When isolated hearts have given short exposure of purified or crude venom toxins, cardiac muscles show considerable increase in contractility [99, 129, 130]. These complex cardiovascular effects by scorpion venom may probably due to direct effect on

vagal and sympathoadrenal stimulation [131-133]. Isolated myocytes show a higher rate of contraction and loss of synchronous activity under the influence of *L. quinquestriatus* venom [133]. Increased deoxyglucose and Ca^{++} uptake into cardiac cells and influx of Ca^{++} into sarcoplasmic changes prevented by pretreatment with propranolol and nifedipine accompany these changes. Further, this stimulation of adrenoreceptors leads to increased influx of Ca^{++} through Ca^{++} channels which then increases contractility [133].

Venoms of all scorpion species affect cardiovascular system and cause pulmonary oedema and cardiac arrhythmias [124]. Venoms also cause cholinergic as well as adrenergic neuron hyper-stimulation by its acting on presynaptic membranes [125, 126]. These venoms have direct effect on gating mechanisms of excitable membranes [134]. As a result there is a massive release of catecholamines from synaptic nerve endings and from adrenal medulla [131, 137]. Elevations of circulating catecholamines and angiotensin result in intense vasoconstriction and cardiac stimulation [138], increased myocardial oxygen requirement and alteration in myocardial perfusions [134, 139]. Several of these mechanisms, together with a possible direct effect of toxin on myocardium may be responsible for myocarditis and focal myocardial necrosis in patients dying from envenomation [140]. Echocardiographic studies have shown severe systolic left ventricular dysfunction following envenomation [141, 142]. This is due to catechol-amine induced metabolic abnormalities in myocardium (138), increased myocardial oxygen requirements [143], myocardial ischemia [139] and direct effect of toxin [130, 140]. Scorpion venoms containing bradykinin-potentiating peptides (hypo-tensive agent) have been found in *L. quinque-striatus*, *T. serrulatus*, *B. martensii* and *B. occi-tanus*. These peptides act as bradykinin-potentiating peptides and can be used as hypotensive agents in the treatment of hypertension. Moraes et al. have reprted that *Tityus bahiensis* scorpion venom modify sodium channel gating to exert hypotension action [144].

The scorpion venom exerts its lethal action by interference with blood coagulation, either by accelerating the process or inhibits the coagulation processes. A peptide with anti-thrombotic action has been reported from *B. martensii* venom [142]. This peptide is related to the resistance against platelet aggregation and increases concentration of prostanglandin I2 in plasma [142]. *T. discrepans* scorpion venom modifies clotting times in humans. *T. discrepans* venom also affects partial thrombo-plastin time, prothrombin time and its direct clotting activity. This venom contains anticoagulant compo-nents which prolong prothrombin time and partial thromboplastic time [143].

7. OTHER PHARMACOLOGICAL ACTIVITIES OF SCORPION VENOM

Scorpion venom is known to modulate kinin pathway in animals. Kinins are peptides generated as a result of the activity of killikrekins (a group of proteolytic enzymes present in most of the tissues and body fluids) on kinogens. Once released, kinins such as bradykinin and related peptides kallikrekin (Lysbradykinin) and Met-Lys-bradykinin produce many physiological responses including pain and hyperanalgesia, in addition to contributing to inflammartory response [144, 145] *M. tamulus* venom causes increased peripheral sympathetic activity with consequent enhancement of adrenergic responses [146]. This venom also causes rhythmic fluctuation in blood pressure producing cardio-vascular collapse and death. It induces spontaneous action potential and causes prolongation of action potential duration in purkinje fibres. This venom enhanced release of acetylcholine and induced repetitive firing of nerve action potentials [147]. This effect may be due to toxins that affect opening of Na^{+} channels in nerve and muscles, which results in increased release of neurotransmitters in peripheral nervous system. It may produce cardiovascular abnormalities and respiratory paralysis also.

Venom of *B. tamulus* causes severe pancreatitis [148], increased osmotic fragility in red blood cells [149, 150], myocarditis [151], hyperglycemia and lipolysis resulting in increased free fatty acids and reduction in triglyceride level [152]. All these cardiovascular, hemodynamic and hematological alternations may be due to massive release of catecholamines, counter-regulatory hormones like glucagons and cortisol [153], angiotensin II [138], thyroxine and triodothyronine

[154] and a reduced insulin secretion [155]. *B. tumulus* venom is found to be protease inhibitors and histamine releasers [40]. Effect of *M. tamulus* and *L. quinquestriatus* venoms on noradrenergic and nitinergic transmission in rat isolated anococcygeus muscle has revealed that both venoms mediate their pharmacological effects via prejunctional mechanism involving activation of voltage sensitive Na^+ channels with consequent release of neurotransmitters mediated by other alpha scorpion toxin [156].

Radha Krishna Murthy and Zare have reported an increase in hemoglobin, mean corpuscular hemoglobin concentration, packed cell volume, plasma hemoglobin levels and increased osmotic fragility of erythrocytes during scorpion envenomation [157]. Fragility of red blood cells has also been observed when incubated with scorpion venom. Rise in packed cell volume and mean corpuscular hemoglobin concentration during scorpion envenomation may be due to hemoconcentration caused by a massive release of catecholamines [158-160] and angitensin II [138]. Phospholipase present in venom could be the agent responsible for increased hemolysis.

Certain venoms such as cobra venom contain phospholipase A, which converts lecithin to lysolecithin, a powerful hemolytic substance [161]. Chhatwal and Habermann have reported presence of phospholipoase A2 in scropion venom [40]. This enzyme is a powerful hemolytic agent and contributes to increased osmotic frgility of red blood cells [154]. Envenomation results in metabolic stress in red blood cells and pumping mechanism failure [140]. A reduction in erythrocyte Na^+K^+ ATPase has been reported in scorpion sting victim [150]. Pande and Mead have observed inhibition of Na^+K^+ATPase activity by elevated free fatty acids through their detergent properties [162]. *Hemiscorpius lepturus* venom increases circulating levels of aspartae aminotransferase, alanine aminotrans-ferase, alkaline phosphatase, creatine phosphokinase and lactic dehydrogenase in rat [163]. Similarly, Omran and Abdel-Rahman have reported elevation in serum glucose, nitrogen, creatine, glutamate oxaloacetate aminotransferase, glutamate-pyruvate aminotransferase, creatine phosphokinase and lactic dehydrogenase, while reduction in serum total protein, uric acid, cholesterol, calcium and potassium [164].

8. SCORPION ANTIVENOM

Scorpion venom is a mixture of many small polypeptides known to induce a strong immunogenic reaction from the host. Potent neurotoxins, which often are relatively small and low abundance molecule, may not always induce production of sufficient quality and quantity of antibody molecules. Therefore, the balance between injected doses, toxicity towards subject animal should be maintained for high quality antibody production. Identification of less abundant but highly potent components in purified mixture and its use as an antigen is highly advantageous in comparison to crude venom to raise antibodies for therapeutic purposes.

Severity of scorpion venom and its rapid diffusion requires appropriate treatment, which must start as soon as possible after sting. Most investigators consider antivenom as the only specific treatment of scorpion stings [127, 165,]. However, others have questioned usefulness of antivenom in eliminating cardiovascular complications of scorpion stings [160, 166].

Use of antivenom in the treatment of scorpion sting was started in 1909; and this mode of therapy, is still the only method used effectively against scorpion stings [167, 168]. Initially scorpion venom extracted from telson homogenate was used as antigen to inject in small doses in horses and sheeps to produce antivenom. After a long period of immunization, the blood of the immunized animal is obtained and the immunoglobulins are purified for use as antivenoms. Demagalhaes has claimed that toxicity of telson extract is less stable than that of pure venom [169]. Crude scorpion venom has many components, which shows poor antigenicity; therefore, other natural chemicals have been added to venom toxins to enhance antigenicity [170]. Scorpion venom is poor in antigenic composition and thus it is difficult to raise antibodies specific to neutralize lethal factor of scorpion venom. However, several attempts have been made to raise species-specific antibodies against scorpion venom. Mohammad et al.have used purified picrate venom obtained from dried telsons to prepare potent antivenom against Egyptean scorpion venom [171].

At Hoffkin Biopharmacutical Corporation Ltd. Mumbai, Kapadia et al. have attempted to prepare anti-scorpion venom serum using grounded telson extract with Freund's incomplete adjuvant [172]. The resulting antiserum, which contains antibodies against scorpion venom, has found to be inefficacious. Kankonkar et al. at Hoffkin Biopharmaceutical Corporation Ltd. Mumbai, India have used Bentonite as adjuvant for extending period of immunization and prepared potent antiserum against *Buthus tamulus* venom capable of neutralizing lethal factors of venom [173].

There are contradictory opinions about effectiveness of scorpion antivenom either in experimental animals or in scorpion sting victims. For quick neutralization of toxic effects of toxins, serotherapy is a well-tested pharmaceutical method that is used for safety of lives of many patients around the world [127, 158, 159, 174, 175]. Contrary to this, Gueron and his co-worker have reported that serotherapy is ineffective [160, 176]. Scorpions usually inject venom into interstitial spaces and not directly into blood circulation. Freire-Maia and Campos have suggested intravenous injection of antivenom to neutralize circulating venom [158]. Moreover, it is likely that antivenom administered intravenously can act on tissues later on. The best result can be achieved when antivenon is administered as early as possible and with adequate quantities to neutralize venom. Radha Kishana Murthy and Zare have reported that species-specific scorpion antiserum prepared at the Hoffkin Biopharmaceutical Corporation Ltd. Mumbai, India, reverses metabolic and hematological alterations caused by *Mesobuthus tamulus* scorpion venom [154].

Due to poor immunogenicity and vast difference in amino acid sequences in active site region, it is very tedious to prepare universal antivenon against scorpion venom. Furthermore, neurotoxic components of scorpion venom are least immunogenic. A recent idea for creating an universal anti-scorpion antivenom is to mix a batch of different anti-scorpion antivenin together to create a universal antivenin but this exposes patients to unnecessary antivenom from other scorpion species which are not from patient's region.

Current method for anti-scorpion antivenom production involves direct injection of crude venom into horses. Besides it, antibodies are also produced from a mixture of a number of scorpion species venoms. However, there are risks associated with injection of antibodies from other animals or passive immunization. The recipient can mount a strong immunologic response to isotype determinants to foreign antibodies. This anti-isotype response can have serious complications because some recipients will produce IgE antibody specific for injected passive antibody. Immune complexes of IgE bound to antibody can mediate systemic mast cell degranulation leading to systemic anaphylaxis. Another possibility is that the recipient will produce IgG or IgM antibodies specific for foreign antibody, which will form complement activating immune complexes. The deposition of these complexes in tissues can lead to type III hypersensitive reaction.

Another approach in neutralization of toxic effects of scorpion stings by serotherapy is possibility of raising antibodies to conserved parts of venom proteins, which could recognize several members of family. Devaux et al. have raised antibodies against an eight residue synthetic polypeptide, which represent conserved region in a set of 25 scorpion toxin sequences [177]. These peptide antibodies have been shown to cross-react with several scorpion toxins belonging to different serotype and neutralize pharmacological effects and biological activities.

Some special antivenoms are also available, which are the same horse antibodies treated with enzymes to produce $F(ab)'_2$ fragments that are used for immunotherapy [178]. Recently smaller recombinant fragments, such as classic monovalent antibody fragments (FAB, scFv and engineered variants: diabodies, triabodies, minibodies and single-domain antibodies) are now engineering as credible alternatives. These fragments retain the targeting specificity of whole antibody and can be used for therapeutic applications [179]. Single-chain Fvs are popular format in which the VH and VL domains are joined with a flexible polypeptide linker preventing dissociation. Antibody Fab and scFv fragments, comprising both VH and VL domains, usually retain the specific, monovalent, antigen binding affinity of the parent IgG, while showing improved pharmacokinetics for tissue penetration [179]. In this context, recently single chain antibodies of human origin have developed

and shown to be effective for neutralization of scorpion toxin envenomation [180-182].

9. SUMMARY

Envenomation of humans by scorpion stings is a serious health problem in some parts of the world. These venoms cause severe systemic inflammation and other complication when injected into humans. Scorpion venoms are mixture of peptides, amines, enzymes and many other bioactive compounds. The most important components, responsible for severe intoxication are short- and long-chain peptides affecting different ion channels (Na^+, K^+, Ca^{++}, Cl^-) either by blocking the channels or modifying their gating properties. They cause abnormal depolarization of the neuronal cells and if not treated on time can lead to death. Fot the neutralization of the venom's induced deleterious effects, venom itself is used for production of antivenom in experimental animals like horse and sheep. Scorpion venoms possess some peptides having antimicrobial, anticancer and insecticidal properties. These make scorpion venom make it an important pharmacological agent in future for developing antimicrobial and antitumour drugs as well as insecticides on commercial scale. Some scorpion venom components have important applications for the treatment of different diseases like autoimmune, cardiovascular and inflammatory diseases.

TRANSPARENCY DECLARATION

The author declares that there is no conflict of interest.

REFERENCES

1. Warrel DA. WHO/SEARO Guidelines for the clinical management of snake bites in the South East Asian regions. J Trop Med Pub Hlth. 1999; 30: 1-85.
2. Briggs DEG. Scorpion takes to the water. Nature. 1987; 326: 645-646.
3. Lourenco WR. Diversity and endenism in tropical versus temperate scorpion communities. Biogeographica. 1994; 70: 155-160.
4. Rochat H, Bernard P, Couraud F. Scorpion toxins: chemistry and mode of action. In: Adv. Cytopharmacol. Ceccarelli F, ed. New York: Raven, 1979: 325-334.
5. Zlotkin E, Eistan M, Bindokas VP, Adams ME, Moyer M, Burkhart W, Fowler E. Functional duality and structural uniqueness of depressant insect selective neurotoxins. Biochem. 1991; 30: 4814-4821.
6. Gordon D, Maskowitz H, Eitan M, Warner C, Catteral, WA, Zlotkin E. Localization of receptor sites for insect selective toxins on Na+ channels by site directed antibodies. Biochem. 1992; 31: 7622-7628.
7. Zlotkin E. In: Athropods venoms. Bettini S, ed. Springer, New York, 1978: 317-369.
8. Inceoglu B, Lango J, Jing J, Chen L, Doymaz F, Pessah IN, Hammock BD. One scorpion, two venoms: Prevenom of *Parabuthus transvaalis* acts as an alternative type of venom with distinct mechanism of action. Proc Natl Acad Sci. 2003; 100: 922-927.
9. Corzo G, Escoubas P, Villegas E, Barnham KJ, He W, Norton RS, Nakazima T. Characterization of unique amphipathic antimicrobial peptides from the venom of the scorpion *Pandinus imperator*. Biochem J. 2001; 359: 35-45.
10. Turkov M, Rashi S, Zilberberg N, Gordon D, Ben Khalifa R, et al. In vitro folding and functional analysis of anti-insect selective scorpion depressant neurotoxin produced in *E. coli*. Proc Express Purific. 1997; 9: 123-131.
11. Balozet L. Venomous invertebrates. In: Venomous animals and their venoms. Vol. 3, Bucherl W, Buckley EE, eds. Academic New York. 1971: 349-371.
12. Keegan HL. Scorpions of medical importance. University Press of Pississippi Jackson. 1980: 43.
13. Brownell P, Polis G. Scorpion biology and research. Oxford University Press, New York, 2001: 3-13.
14. Polis G. The biology of scorpions. Stanford University Press, Stanford, 1990: 247-193.
15. Possani LD, Becerril B, Delepierre M, Tytgat J. Scorpion toxin specific for Na^+ channel. Eur J Biochem. 1999; 264: 287-300.
16. Becceril B, Marangoni S, Possani LD. Toxin and genes isolated from the scorpion of the genus *Tityus*: a review. Toxicon. 1997; 35: 821-835.
17. Nakagawa Y, Lee YM, Lehmberg E, Herrmann R, Maskowitz H, Jones AD, Hammock BD. Antiscorpion toxin 5 (AaIT5) from *Androctonus australis*. Eur J Biochem. 1997; 246: 496-501.

18. Murugesan S, Murthy RKK, Noronha OPD, Samuel AM. Tc99m-scorpion venom: labeling, biodistribution and scintimaging. J Venom Anim Toxin. 1999; 5: 35-46.
19. Ismail M. The scorpion-envenoming syndrome. Toxicon. 1995; 33: 825-858.
20. Albert D, Rama MB, Karen W, Eddie C. Expression and secretion of a functional scorpion insecticidal toxin in cultured mouse cells. Biotechnol. 1990; 8: 339-342.
21. Stewart LMD, Hirst M, Freber ML, Merryweather AT, Clayley PJ, Posse RD. Construction of an improved baculovirus insecticide containing an insect specific toxin gene. Nature. 1991; 352: 85-88.
22. Mc Cutchen BF, Hammock BD. Recombinant baculovirus expressing an insect-selective neurotoxin. In: Natural and engineered pest management agent. Hedin PA, Menn JJ, eds. Hallingworth RM, 1994: 348-367.
23. Pelhate M, Stankiewicz M, Ben Khalifa R. Anti-insect scorpion toxins: historical account, activities and prospects. CR Seances Soc Biol Fil. 1998; 192: 463-484.
24. Karbat I, Frolow F, Froy O, Gilles N, Cohen L, Turkov M, et al. Molecular basis of the high insecticidal potency of scorpion α toxins. J Biol Chem. 2004; 279: 31679-31686.
25. Gurevitz M, Karbat I, Cohen L, Ilan N, Kahn R, Turkov M, et al. The insecticidal potential of scorpion β-toxins. Toxicon. 2007; 49: 473-489.
26. Gordon D, Karbat I, Ilan N, Cohen L, Kahn R, Gilles N, et al. The differential preference of scorpion α-toxins for insect or mammalian sodium channels: implications for improved insect control. Toxicon. 2007; 49: 452-472.
27. Pelhate M, Zlotkin E. Action of insect toxin and other toxins derived from the venom of the scorpion *Androctonus australis* on isolated giant axons of the cockroach (*Periplanata americana*). J Exp Biol. 1982; 97: 67-77.
28. Ben Khalifa R, Stankiewics M, Lapied B, Turkov M, Zilberberg N, Gurevitz M, Pelhate M. Refined electrophysiological analysis suggests that a depressant toxin is a sodium channel opener rather than a blocker. Life Sci. 1997; 61: 819-830.
29. Wang GK, Strichartz GR. Purification and physiological characterization of neurotoxins from the venoms of the scorpion *Centruroides sculpturatus* and *Leiurus quinquestriatus*. Mol Pharmacol. 1983; 23: 519-533.
30. Bontems F, Roumestand C, Gilquin B, Menez A, Toma F. Refined structure of charybdotoxin: common motifs in scorpion toxins and insect defensins. Science. 1991; 254: 1521-1523.
31. Crest M, Jacquet G, Gola M, Zerreouk H, Benslimane A, Rochat H, et al. Kaliotoxin, a novel peptidyl inhibitor of neuronal 13 K-type Ca^{++}-activated K^{+} channel characterized from *Androctonus mauretanicus mauretanicus* venom. J Biol Chem. 1992; 267: 1640-1647.
32. Zlotkin A, Fishman Y, Elazar M. AaIT: from neurotoxin to insecticide. Biochiemie. 2000; 82: 869-881.
33. Higgs S, Olson KE, Klimowski L, Powers AM, Carlson JO, Possee RD, Beaty BJ. Mosquito sensitivity to a scorpion neurotoxin expressed using an infectious Snindbis virus vector. Insect Mol Biol. 1995; 4: 97-103.
34. Loret EP, Martin-Eauclaire MF, Mansuelle P, Sampieri F, Granier C, Rochat H. An anti-insect toxin purified from the scorpion *Androctonus astralis* Hector also acts on the α and β sites of the mammalian sodium channels sequence and circular dichroism study. Biochem. 1991; 30: 633-640.
35. Eitan M, Fowler E, Herrmann R, Duval A, Pelhate M, Zlotkin E. A scorpion venom neurotoxin paralytic to insects that affects sodium current inactivation: purification, primary structure and mode of action. Biochem. 1990; 29: 5941-5947.
36. Krimm I, Gilles N, Sautire P, Stankiewicz M, Pelhate M, Gordon D, Lancelin JM. NMR structures and activity of a novel α-like toxin from the scorpion *Leiurus quinquestriatus* hebraeus. J Mol Biol. 1999; 285: 1749-1763.
37. Dhawan R, Joseph S, Sethi A, Lala AK. Purification and characterization of a short insect toxin from the venom of the scorpion *Buthus tamulus*. FEBS Lett. 2003; 528: 261-266.
38. Gawade SP. Excitatory effects of Buthus C56 *Drosophila* on larval neuromuscular junction. J Venom Anim Toxin Incl Trop Dis. 2003; 9(1): 65-75.
39. Gershburg E, Stockholm D, Froy O, Rashi S, Gurevitz M, Chejanovsky N. Baculovirus mediated expression of a scorpion depressant toxin improve the insecticidal efficacy achieved with excitatory toxin. FEBS Lett. 1998; 422: 132-136.
40. Chhatwal GS, Habbermann E. Neurotoxins, protease inhibitors and histamine releasers in the venom of the red scorpion (*Buthus tamulus*): isolation and partial characterization. Toxicon. 1981; 19: 807-823.

41. Lala K, Narayanan P. Purification, N-terminal sequence and structural characterization of a toxin protein from the Indian scorpion venom, *Buthus tamulus*. Toxicon. 1994; 32: 325-338.
42. Wudayagiri R, Inceoglu B, Herrmann R, Choudhary MD, Hammock BD. Isolation and characterization of a novel lepidopteran-selective toxin from the venom of the South Indian scorpion, *Mesobuthus tamulus*. BMC Biochem. 2001; 2: 11-16.
43. Steiner H, Hultmark D, Engstrom A, Bennich H, Boman HG. Sequence and specificity of two antimicrobial proteins involved in insect immunity. Nature. 1981; 292: 246-248.
44. Bulet P, Cociancich S, Dimarcq JL, Lambert J, Reichhart JM, Hoffmann D, et al. Insect immunity: Isolation from a coleopteran insect of novel inducible antibacterial peptide and of new members of the insect defensin family. J Biol Chem. 1991; 266: 24520-24525.
45. Nicolas P, Mor A. Peptides as weapons against microorganisms in the chemical defense system of vertebrate. Annu Rev Microbiol. 1995; 49: 277-304.
46. Hoffman JA, Kafatos FC, Janeway CA, Ezekowitz RA. Phylogenetic perspective in innate immunity. Science. 1999; 284: 1313-1318.
47. Fennel JF, Shipman WH, Cole LJ. Antibacterial action of melittin, a polypeptide from bee venom. Proc Soc Exp Biol Med. 1968; 127: 707-710.
48. Krishnakumari V, Nagaraj R. Antimicrobial and hemolytic activities of crabrolin, a 13-residue peptide from the venom of the European hornet, *Vespa crabro*, and its analogs. J Pept Res. 1997; 50: 88-93.
49. Yan L, Adams ME. Lycotoxins, antimicrobial peptides from the venom of the wolf spider *Lycosa carolinensis*. J Biol Chem. 1998; 273: 2059-2066.
50. Hwang PM, Vogel HJ. Structure-function relationship of antimicrobial peptides. Biochem Cell Biol. 1998; 76: 235-246.
51. Larrick JW, Wright SC. Cationic antimicrobial peptides. Drug Future. 1996; 21: 41-48.
52. Hancock RE, Lehrer R. Cationic peptides: a new source of antibiotics. Trends Biotechnol. 1998; 16: 82-88.
53. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002; 415(6870): 389-395.
54. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti infective therapeutic strategie. Nature Biotechnol. 2007; 24(12): 1551-1557.
55. Dai L, Yasuda A, Naoki H, Corzo G, Andriantsiferana M, Nakajima T. IsCT, a novel cytotoxic linear peptide from scorpion *Opisthacanthus madagascariensis*. Biochem Biophys Res Commun. 2001; 286: 820-825.
56. Arpornsuwan T, Buasakul B, Jaresitthikunchai J, Roytrakul S. Potent and rapid antigonococcal activity of the venom peptide BmKn2 and its derivatives against different Maldi biotype of multidrug-resistant *Neisseria gonorrhoeae*. Peptides. 2014; 53: 315-320.
57. Epad RM, Vogel HJ. Diversity of antimicrobial peptides and their mechanism of action. Biochim Biophys Acta. 1999; 1462: 11-28.
58. Mousli M, Bueb JL, Bronner C, Rouot B, Landry Y. G-protein activation: a receptor independent mode of action for cationic amphipathic neuropeptides and venom peptides. Trends Pharmacol Sci. 1990; 11: 358-362.
59. Bulet P, Hetru C, Dimarcq J, Hoffmann D. Antimicrobial peptides in insects: structure and function. Dev Comp Immunol. 1999; 23: 329-344.
60. Cociancich S, Goyffon M, Bontems F, Bulet P, Bouet F, Menez A, Hoffmann J. Purification and characterization of a scorpion defensin, a 4 kD antimicrobial peptide presenting structural similarities with insect defensins and scorpion toxins. Biochem Biophys Res Commun. 1993; 194: 17-22.
61. Ehret-Sabatier L, Loew D, Goyffon M, Fehlbaum P, Hoffman JA, Van Dorsselaer A, Bulet P. Characterization of novel cyteine rich antimicrobial peptides from scorpion blood. Biochem Mol Biol. 1996; 271: 29537-29544.
62. Hetru C, Letellier L, Oren Z, Hoffmann JA, Shai Y. Androctonin, a hydrophilic disulphide-bridged non-haemolytic anti-microbial peptide: a plausible mode of action. Biochem J. 2000; 345: 653-664.
63. Moerman L, Bosteels S, Noppe W, Willems J, Clynen E, Schoofs L. Antibacterial and antifungal properties of alpha-helical, cationic peptides in the venom of scorpions from southern Africa. Eur J Biochem. 2002; 269: 4799-4810.
64. Powers JP, Hancock RE. The relationship between peptide structure and antibacterial activity. Peptides. 2003; 24: 1681-1691.
65. Ramírez-Carreto S, Jiménez-Vargas JM, Rivas-Santiago B, Corzo G, Possani LD, Becerril B. Peptides from the scorpion *Vaejovis punctatus* with broad antimicrobial activity. Peptides. 2015; 73: 51-59.

66. Bao A, Zhong J, Zeng XC, Nie Y, Zhang L, Peng ZF. A novel cysteinefree venom peptide with strong antimicrobial activity against antibioticsresistant pathogens from the scorpion *Opistophthalmus glabrifrons*. J Pept Sci. 2015; 21: 758-764.
67. de Melo ET, Estrela AB, Santos EC, Machado PR, Farias KJ, Torres TM. Structural characterization of a novel peptide with antimicrobial activity from the venom gland of the scorpion *Tityus stigmurus*: stigmurin. Peptides. 2015; 68: 3-10.
68. Bandyopadhyay S, Junjie RL, Lim B, Sanjeev R, Xin WY, Yee CK. Solution structures and model membrane interactions of Ctriporin, an anti-methicillin-resistant *Staphylococcus aureus* peptide from scorpion venom. Biopolymers. 2014; 101: 1143-1153.
69. Li Z, Xu X, Meng L, Zhang Q, Cao L, Li W. Hp1404, a new antimicrobial peptide from the scorpion *Heterometrus petersii*. PLoS One. 2014; 9: 97539.
70. Conde R, Zamudio F Z, Rodriguez MH, Possani LD. Scorpine, an antimalarial and antibacterial agent purified from scorpion venom. FEBS Lett. 2000; 471: 165-168.
71. Torres-Larios A, Gurrola GB, Zamudio FZ, Possani LD. Hadrurin, a new antimicrobial peptide from the venom of the scorpion *Hadrurus aztecus*. Eur J Biochem. 2000; 267: 5023-5031.
72. Verdonck F, Bosteel S, Desmet J, Moerman L, Noppe W, Willems J, et al. A novel class of pore forming peptides in the venom of the *Parabuthus schlechteri* Purcell (Scorpions: Buthidae). Cinebasia. 2000; 16: 247-260.
73. White SH, Wimley WC, Selsted ME. Structure, function and membrane integration of defensins. Curr Opin Struct Biol. 1995; 5: 521-527.
74. Matsuzaki K. Magainins as paradigm for the mode of action of pore forming peptides. Biochim Biophys Acta. 1998; 1376: 391-400.
75. Du Q, Hou X, Wang L, Zhang Y, Xi X, Wang H, et al. AaeAP1 and AaeAP2: novel antimicrobial peptides from the venom of the scorpion, *Androctonus aeneas*: structural characterisation, molecular cloning of biosynthetic precursor-encoding cDNAs and engineering of analogues with enhanced antimicrobial and anticancer activities. Toxins. 2015; 7: 219-237
76. Al-Asmari AK, Alamri MA, Almasoudi AS, Abbasmanthiri R, Mahfoud M. Evaluation of the in vitro antimicrobial activity of selected Saudi scorpion venoms tested against multidrug-resistant microorganisms. J Global Antimicrob Resist. 2007; 10: 14-18.
77. Sikora K. Cancer survival in Britain in poorer than that of her comparable European neighbours. BMJ. 1999; 319: 461-462.
78. De Carvalho DD, Schmitmeier S, Novello JC, Markland FS. Effect of BJcuL (a lectin from the venom of the snake *Bothropos jararacussu*) on adhesion and growth of tumor and endothelial cells. Toxicon. 2001; 39: 1471-1476.
79. Chiang HS, Swaim MW, Huang TF. Characterization of platelet aggregation induced by human breast carcinoma and its inhibition by snake venom peptide, trigramin and rhodostomin. Breast Cancer Res Treat. 1995; 33: 225-235.
80. Maristela P, Daniela DC, Antonio RG, Delwood CC. The effect of lectin from the venom of the snake, *Bathrops jararacussu*, on tumor cell proliferation. Anticancer Res. 1999; 19: 4023-4026.
81. Zhou Q, Sherwin RP, Parrish C, Richters V, Groshen SG, Tsao-Wei D, Markland FS. Contortrostatin, a dimmer disintegrin from *Agkistrodon contortrix contortrix*, inhibits breast cancer progression. Breast Cancer Res Treat. 2000; 61: 249-260.
82. Yarom R, Braun K. Myocardiopathology following scorpion venom injection. Isr J Med Sci. 1969; 5: 849-852.
83. Tarasiuk A, Khvatskin S, Sofer S. Effect of antivenom serotherapy on haemodynamic patho-physiology in dogs injected with of *Leiurus quinquestriatus* scorpion venom. Toxicon. 1998; 36: 963-971.
84. Omran MAA. In vitro anticancer effect of scorpion *Leiurus quinquestriatus* and Egyptian cobra venom on human breast and prostate cancer cell lines. J Med Sci. 2003; 3: 66-86.
85. Bruses JL, Capaso J, Katz E, Pilar G. Specific in vitro biological activities of snake venom myotoxins. J Neurochem. 1993; 60: 1030-1042.
86. Deshane J, Garner CC, Sontheimer H. Chlorotoxin inhibits glioma invasion via matrix metallo-proteinase-2. J Biol Chem. 2003; 278: 4135-4144.
87. Jager H, Dreker T, Buck A, Giehl K, Gress T, Grissmer S. Blockage of intermediate-conductance Ca^{2+} activated K^+ channels inhibit human pancreatic cancer cell growth in vitro. Mol Pharmacol. 2004; 65: 630-638.
88. Gupta SD, Gomes AN, Debnath A, Saha A, Gomes AP. Apoptosis induction in human leukemic cells by a novel protein Bengalin, isolated from Indian black

scorpion venom: through mitochondrial pathway and inhibition of heat shock proteins. Chem Biol Interact. 2010; 183: 293-303.

89. Al-Asmari AK, Islam M, Al-Zahrani AM. In vitro analysis of the anticancer properties of scorpion venom in colorectal and breast cancer cell lines. Oncol Lett. 2016; 2: 1256-1262.
90. Gupta SD, Debnath A, Saha A, Giri B, Tripathi G, Vedasiromoni JR, et al. Indian black scorpion (*Heterometrus bengalensis* Koch) venom induced antiproliferative and apoptogenic activity against human leukemic cell lines U937 and K562. Leuk Res. 2007; 31: 817-825.
91. Zhang YY, Wu LC, Wang ZP, Wang ZX, Jia Q, Jiang GS, Zhang WD. Antiproliferation effect of polypeptide extracted from scorpion venom on human prostate cancer cells in vitro. J Clin Med Res. 2009; 1: 24-31.
92. Zargan J, Sajad M, Umar S, Naime M, Ali S, Khan HA. Scorpion (*Androctonus crassicauda*) venom limits growth of transformed cells (SH-SY5Y and MCF-7) by cytotoxicity and cell cycle arrest. Exp Mol Pathol. 2011; 91: 447-454.
93. Almaaytah A, Albalas Q. Scorpion venom peptides with no disulfide bridges: a review. Peptides. 2014; 51: 35-45.
94. Roger JC, Qu Y, Tanada TN, Scheur T, Catterall WA. Molecular determinants of high affinity binding of alpha-scorpion toxin and Sea anemone toxin in S3-S4 extracellular loop in domain IV of the Na+ channel alpha-subunit. J Biol Chem. 1996; 271: 15950-15962.
95. Cestele S, Caterall WA. Molecular mechanism of neurotoxic action on voltage-gated sodium channels. Biochemie (Paris). 2000; 82: 883-892.
96. Martin-Eauclaire MF, Couraud F. Scorpion neurotoxins: effects and mechanism. In: Hand book of neurotoxicology. Chang LW, Dyer RS, eds Marcel Dekker New York, 1995: 683-716.
97. Gwee MC, Nirthanan S, Khoo HE, Gopalkrishnakone P, Kini RM, Cheah LS. Autonomic effect of some scorpion venoms and toxins. Clin Exp Pharmacol Physiol. 2002; 29: 795-801.
98. Couraud F, Jover E, Dubois JM, Rochat H. Two types of scorpion toxin receptor sites, one related to the activation, the other to the inactivation of the action potential Na+ channel. Toxicon. 1982; 20: 9-16.
99. Couto AS, Moreaes-Santos T, Azevedo AD, Almeida AP, Freire-Maia L. Effect of Tsgamma, purified from *Tityus serrulatus* scorpion venom, on the isolated rat atria. Toxicon. 1992; 30: 339-343.
100. Yatani A, Kirsh GE, Possani LD, Brown AM. Effects of New World ecorpion toxins on single channel and whole cell cardiac sodium currents. Am J Physiol. 1988; 254: 443-451.
101. Romi-Labrun R, Martin-Eauclaire MF, Escoubas P, Wu FQ, Lebrun B, Hisada M, Nakazima T. Characterization of four toxins from *Buthus martensi* scorpion venom, which act on apaminesensitive Ca^{++}-activated K^{+} channels. Eur J Biochem. 1997; 245: 457-464.
102. D' Ajellow A, Zlotkin E, Miranda F, Lissitzky. The effect of the scorpion venom and pure toxin on the cockroach nervous system. Toxicon. 1972; 10: 399-404.
103. Miller C, Moczydlowski E, Latore R, Philips M. Charybdotoxin, a potent inhibitor of single Ca^{++}-activated K^{+} channels from mammalian skeleton muscle. Nature. 1985; 313: 316-318.
104. Miller C. The charybdotoxin family of K^{+} channel blocking peptides. Neurons. 1995; 15: 5-10.
105. Hoffmann JA, Hetru C. Insect defensins: inducible antimicrobial peptides. Immunol Today. 1992; 13: 411-415.
106. Bablito J, Jover E, Couraud F. Activation of the voltage sensitive sodium channel by a β-scorpion toxin in the rat brain nerve ending particles. J Neurochem. 1986; 37: 1763-1770.
107. Garcia CM, Leonard RJ, Novik J, Stevens SP, Schmalhofer W. Purification, characterization and biosynthesis of margaritatus venom that selectively inhibits voltage dependent potassium channel. J Biol Chem. 1993; 689(25): 18866-18874.
108. Lin CS, Boltz RC, Blake JT, Nguyen M, Talento A, Fischer PA, et al. Volatge-gated potassium channels regulate calcium dependent pathways involved in human T-lymphocytes activation. J Exp Med. 1993; 177: 637-645.
109. Bednarek MA, Bugianesi RM, Leonard RJ, Felix JP. Chemical synthesis and structurefunction studies of margtoxin, a potent inhibitor of voltage-dependent potassium channel in human T-lymphocytes. Biocem Biophys Res Commun. 1994; 198: 619-625.
110. Garcia ML, Garcia-Calvo M, Hidalgo P, Lee A, Mac Kinnon R. Purification and characterization of three inhibitors of voltage dependent K+ channels from *Leiurus quinquestriatus* var. hebraeus venom. Biochem. 1994; 33: 6834-6839.

111. Martins JC, Van JC, Borremanus FA. Determination of the three-dimensional solution structure of the scyllatoxin by 1H NMR. J Mol Biol. 1995; 253: 590-603.

112. Pappone PA, Chalan MD. *Pandinus imperator* scorpion venom blocks voltage-gated K+ channel in nerve fibers. J Neurosci. 1987; 7: 3300-3305.

113. Sands SB, Lewis RS, Chalan MD. Charbdotoxin blocks voltage-gated K^+ channel in human and murine T-lymphocytes. J Gen Physiol. 1989; 93: 1061-1074.

114. Valdivia H, Kirby MS, Lederer WJ, Coronado R. Scorpion toxins targated against the sarcoplasmic reticulum Ca^{++} release channel of skeletal and cardiac muscle. Proc Nat Acad Sci USA. 1992; 89: 12185-12189.

115. el Havek R, Lokuta AJ, Arevalo C, Valdivia HH. Peptide probe of ryanodine receptor function. Imperatoxin A, a peptide from the venom of the scorpion *Pandinus imperator*, selectively activates skeletaltype ryanodine receptor isoforms. J Biol Chem. 1995; 270: 28696-28704.

116. Pappone PA, Lucero MT. *Pandinus imperator* scorpion venom blocks voltage gated potassium channels in GH3 cells. J Gen Physiol. 1988; 91: 817-833.

117. Galvez A, Gimenez-Gallego G, Ruben JP, Rov-Contancin L, Feigenbaun P, Kaczorowski GJ, Garcia ML. Purification and characterization of a unique peptidyl probe for the high conductance calcium-activated potassium channel from the venom of the scorpion *Buthus tamulus*. J Biol Chem. 1990; 265: 11083-11090.

118. Marangoni S, Ghiso J, Sampaio SV, Arantes EC, Giglio JR, Oliviera B, Frangione B. The complete amino acid sequence of toxin TsTX-VI isolated from the venom of the scorpion *Tityus serrulatus*. J Prot Chem. 1990; 9: 595-601.

119. Grishin EV, Korolkova YV, Kozlov KA, Lipkin AV, Nosyreva ED, Pluzhnikov KA, et al. Structure and function of potassium channel inhibitor from black scorpion venom. Pure Appl Chem. 1996; 68: 2105-2109.

120. More SS, Mirajkar KK, Gadag JR, Menon KS, Mathew MK. A novel Kv1.1 potassium channel-blocking toxin from the venom of *Palamneus gravimanus* (Indian black scorpion). J Venom Anim Toxin Incl Trop. 2005; 11(3): 315-335.

121. De Bin JA, Maggio JE, Strichartz GR (1993) Purification and characterization of chlorotoxin, a Clchannel ligand from the venom of the scorpion. Am J Physiol Cell Physiol 264:361-369.

122. Lippens G, Najib S, Wodak J, Tartar A. Sequential assignment and solution structure of chlorotoxin, a small peptide from scorpion that blocks chloride channel. Biochem. 1995; 34: 13-21.

123. Zamudio FZ, Conde R, Arevalo C, Becerril B, Martin BM, Valdivia HH, Possani LD. The mechanism of inhibition of ryanodine receptor channel by imperotoxin I, a heterodimeric protein from the scorpion *Pandius imperator*. J Biol Chem. 1997; 272: 11886-11894.

124. Zamudio FZ, Gurrola GB, Arvalo C, Sreekumar R, Walker JW, Valdivia HH, Possani LD. Primary structure and synthesis of imperotoxin A (Iptxa), a peptide activator of Ca^{++} release channels/rynodine receptors. FEBS Lett. 1997; 405: 385-389.

125. Chuang RSI, Jaffe H, Cribbs L, Perez-Reyes EJ, Stwartz KJ. Inhibition of T-type voltagegated calcium channels by a new scorpion toxin. Natl Neuro Sci. 1998; 1: 668-674.

126. Freire-Maia L, Pinto GI, Franco I. Mechanism of the cardiovascular effects produced by purified scorpion toxin in the rat. J Pharmacol Exp Ther. 1974; 188: 207-213.

127. Ismail M. The scorpion-envenoming syndrome. Toxicon. 1995; 33: 825-858.

128. Tarasiuk A, Janco J, Sofer S. Effect of scorpion venom on central and peripheral circulatory response in an open-chest dog model. Acta Physiol Scand. 1997; 161: 141-146.

129. Ismail M, Osmon OH, Gumma KA, Karrar MA. Some pharmacological studies with scorpion (*Pandinus exitiallis*) venom. Toxicon. 1974; 2: 75-82.

130. Almeida AP, Alpoim NC, Freire-Maia L. Effects of purified scorpion toxin (*Tityus* toxin) on the isolated guinea pig heart. Toxicon. 1982; 20: 855-865.

131. Moss J, Kajik T, Henery DP, Kopin IJ. Scorpion venom induced discharge of catecholamines accompanied by hypertension. Brain Res. 1973; 54: 381-385.

132. Freire-Maia L, Campos JA. Pathophysiology and treatment of scorpion poisoning. In: Ownby LC, Odel GV, eds. Natural toxins, characterizations, pharmacology and therapeutics. Pergamon Press Oxford, 1989: 139-159.

133. Tarasiuk A, Sofer S. Effect of adrenergic blockade and ligation of spleen vessels on haemodynamics of dogs injected with scorpion venom. Crit Care Med. 1999; 27: 365-372.

134. Sofer S. Scorpion envenomation. Int Care Med. 1995; 21: 627-628.

135. Ramchandran LK, Agrawal OP, Achyutan KE, Chudhary L, Vedasiromani JR, Ganguli DK. Fractionation and biological activities of the venom of the Indian scorpions *Buthus tamulus* and *Heterometrus bengalensis*. Ind J Biochem Biophys. 1986; 23: 355-358.

136. Russel FE. Toxic effects of animal toxins. In: Idaassen CD, Amdur MO, Doull J, eds. Toxicology basic science of poisons. 3rd edn, New York: Macmillan, 1986: 706-756.

137. Henriques MC, Gassinelli G, Diniz CR, Gomez MV. Effect of the venom of the scorpion *Tityus serrulatus* on adrenal gland catecholamines. Toxicon. 1968; 5: 175-179.

138. Radha Krishna Murthy K, Vakil AE. Elevation of plasma angiotensin level in dogs by Indian red scorpion (*Buthus tamulus*) venom and its reversal by administration of insulin and tolazoline. Indian J Med Res. 1988; 88: 376-379.

139. Margulis G, Sofer S, Zalstein E, Zucker N, Ilia R, Gueron M. Abnormal coronary perfusion in experimental scorpion envenomation. Toxicon. 1994; 32: 1675-1678.

140. Wang R, Moreau P, Deschamps A, de Champlain J, Sauve R, Foucart S, et al.Cardiovascular effects of *Buthus martensi* (Karsch) scorpion venom. Toxicon. 1994; 32: 191-200.

141. Amaral CFS, Lopes JA, Magalhaes RA, de Rezende NA. Electrocardiographic, enzymatic and echocardiographic evidence of myocardial damage after *Tityus serrulatus* scorpion poisoning. Am J Cardiol. 1991; 67: 655-657.

142. Abroug E, Ayari M, Nouria S, Gamra H, Boujdaria R, Elatrons S, et al. Assessment of left ventricular function in severe scorpion envenomation: combined haemodynamic and echodopller study. Int Care Med. 1995; 21: 629-635.

143. Gueron M, Adolf RJ, Grupp IL, Gabel M, Grupp G, Fowler NO. Haemodynamic and myocardial consequences of scorpion venom. Am J Cardiol. 1980; 45: 1979-1986.

144. Couture R, Harrisson M, Vianna RM, Cloutier F. Kinin receptors in pain and inflammations. Eur J Pharmacol. 2001; 429: 161-176.

145. Campbell DJ, Dixon B, Kladis A, Kemme M, Santmaria, JD. Activation of the kallikrein-kinin system by cardiopulmonary bypass in humans. Am J Physiol. 2001; 281: 1059-1070.

146. Gwee MC, Cheah LS, Nirthanan S, Gopalkrishnakone P, Wang PT. Pre-junctional action of the venom from the Indian red scorpion *Mesobuthus tamulus* on adrenergic transmission in vitro. Toxicon. 1994; 32: 201-209.

147. Rowan EG, Vatanpour H, Furman BL, Harvey AL, Tanira MO, Gopalkrishnakone P. The effect of Indian red scorpion Buthus tamulus venom in in vivo and in vitro. Toxicon. 1992; 30: 1157-1164.

148. Murthy RKK, Medh JD, Dave BN, Vakil YE, Billimoria FR. Acute pancreatitis and reduction of H^+ ion concentration in gastric secretions in experimental acute myocarditis produced by Indian red scorpion (*Buthus tamulus*) venom. Indian J Exp Biol. 1989; 27: 242-244.

149. Murthy RKK, Hossein Z. Increased osmotic fragility of red cells of incubation at 37^0C for 20 hours in dogs with acute myocarditis produced by scorpion (*Buthus tamulus*) venom. Indian J Exp Biol. 1986; 38: 206-210.

150. Murthy RKK, Anita AG, Dave BN, Billimoria FR. Erythrocyte Na^+K^+ATPase activity inhibition and increased red cell fragility in experimental myocarditis produced by red scorpion (*Buthus tamulus*) venom. Indian J Med Res. 1988; 88: 536-540.

151. Murthy RKK, Yeolekar ME. Electrocardiographic changes in acute myocarditis produced by the scorpion (*Buthus tamulus*) venom. Indian Heart J. 1986; 38: 206-210.

152. Murthy RKK, Hossein Z, Medh JD, Kudalkar JA, Yeolekar ME, Pandit SP, et al. Disseminated intravascular coagulation and disturbances in carbohydrate and fat metabolism in acute myocarditis produced by Indian red scorpion (*Buthus tamulus*) venom. Indian J Med Res. 1988; 87: 318-325.

153. Murthy RKK, Haghanzari L. The blood level of glucagon, cortisol, and insulin following the injection of venom by the scorpion (*Mesobuthus tamulus*, Pocock) in dogs. J Venom Anim Toxin. 1999 5: 200-219.

154. Murthy RKK, Zare A. Effect of Indian red scorpion (*Mesobuthus tamulus concanesis*, Pocock) venom on thyroxine and triiodothyronine in experimental acute myocarditis and its reversible by species-specific antinenom. Indian J Exp Biol. 1998; 36: 16-21.

155. Murthy RKK, Anita AG. Reduced insulin secretion in acute myocarditis produced by scorpion (*Buthus tamulus*). Indian Heart J. 1986; 38: 467-469.

156. Gwee MC, Nirthanan S, Khoo HE, Gopalkrishnakone P, Kini RM, Cheah LS. Autonomic effect of some scorpion venoms and toxins. Clin Exp Pharmacol Physiol. 2002; 29: 795-801.

157. Murthy RKK, Zare A. The use of antivenin reverses hematological and osmotic fragility changes of erthrocytes caused by Indian red scorpion *Mesobuthus tamulus concanesis*, Pocock in experimental envenoming. J Venom Anim Toxins. 2001; 7: 113-138.
158. Freire-Maia L, Campos JA. On the treatment of the cardiovascular manifestations of scorpion envenomation. Toxicon. 1987; 25: 125-130.
159. Freire-Maia L, De Matos IM. Heparin or a PAF antagonist (BN-52021) prevents the acute pulmonary oedema induced by *Tityus serrulatus* scorpion venom on the rat. Toxicon. 1993; 31: 1207-1210.
160. Gueron M, Ovsyshcher I. What is the treatment for the cardiovascular manifestations of scorpion envenomation? Toxicon. 1987; 25: 121-124.
161. Best CH, Taylor NBA. Textbook in applied physiology of medical practice. Baltimore: Williams and Wilkins, 1967.
162. Pande SV, Mead JF. Inhibition of enzyme activities by free fatty acids. J Biol Chem. 1968; 243: 6180-6186.
163. de Rezende NA, Dias MB, Campolina D, Chavez-Olortegui C, Diniz C, Amaral CFS. Efficacy of antivenom therapy for neutralizing venom antigen in patients stung by *Tityus serrulatus* scorpion. Am J Trop Med Hyg. 1995; 52: 277-280.
164. Omran MAA, Abdel-Rahman MS. Effect of the scorpion *Leiurus quinquestriatus* (H&E) venom on the clinical chemistry parameters of the rat. Toxicol Lett. 1992; 61: 99-109.
165. Ghalim N, El-Hafny B, Sebti F, Heikel J, Lezar N, Moustanir R, Benslimane A. Scorpion venom and serotherpy in Morocco. Am J Trop Med Hyg. 2000; 62: 277-283.
166. Sofer S, Shahak E, Gueron M. Scorpion envenomation and antivenom therapy. J Pediatr. 1994; 124: 973-978.
167. Balozet L. Scorpionism in the Old World. In: Bücherl W, Buckley E, eds. Venomous animals and their venoms. New York: Academic Express, 1971: 349-371.
168. Theakston RD, Warrell DA, Grifithis E. Report of a WHO workshop on the standardization and control of antivenoms. Toxicon. 2003; 41: 541-557.
169. Demagalhaes O. Scorpionism. J Trop Med Hyg. 1938; 41: 393-399.
170. Balozet L. Scorpion venoms and anti-scorpion serum. In: Venom. Buckley EE, Porges N, eds. Washington DC. Public. No. 44. Am Adv Sci. 1956.
171. Mohammed AH, Darwish MA, Honi Ayobe M. Immunological studies on scorpions (*Leiurus quinquestriatus*) antivenin. Toxicon. 1975; 13: 67-68.
172. Kapadia ZS, Master RWP, Rao SS. Immunological studies in telson extracts of Indian and Egyptian scorpion venom. Indian J Exp Biol. 1964; 2: 75-77.
173. Kankonakar RC, Kulakarni DG, Hulikavi CB. Preparation of a potent anti-scorpion venom serum against the venom of red scorpion (*Buthus tamulus*). J Postgrad Med. 1998; 44: 85-92.
174. Amaral CFS, Dias MB, Campolina D, Proietti FA, de Rezende NA. Childrens with adrenergic manifestation of envenomation after Tityus serrulatus scorpion sting are protected from early anaphylactic antivenom reaction. Toxicon. 1994; 32: 211-215.
175. Amaral CFS, de Rezende NA. Both cardiogenic and noncardiogenic factors are involved in the pathogenesis of pulmonary oedema after scorpion envenoming. Toxicon. 1997; 35: 997-998.
176. Gueron M, Marquilis G, Sofer S. Echocardiographic and radionucleid angiographic observations following scorpion envenomation by *Leiurus quinquestriatus*. Toxicon. 1990; 28: 1005-1009.
177. Devaux C, Fourquet P, Granier C. A conserved sequence region of scorpion toxin rendered immunogenic induces broadly cross-reactive, neutralizing antibodies. Eur J Biochem. 1996; 242(3): 727-735.
178. Espino-Solis GP, Riano-Umbarila L, Becerril B, Possani LD. Antidotes against venomous animals: state of the art and prospectives. J Proteomics. 2009; 72(2): 183-199.
179. Holliger PH, Hudson JP. Engineering antibody fragments and the rise of single domains. Nat Biotechnol. 2005; 23: 1126-1136.
180. Riaño-Umbarila L, Contreras-Ferrat G, Olamendi-Portugal T, Morelos-Juárez C, Corzo G, Possani LD, Becerril B. Exploiting cross-reactivity to neutralize two different scorpion venoms with one single chain antibody fragment. J Biol Chem. 2011; 286: 6143-6151.
181. Canul-Tec J-C, Riaño-Umbarila L, Rudinño-Pinera E, Becerril B, Possani LD, Torres-Larios A. Structural basis of neutralization of the major toxic component from the scorpion *Centruroides noxius* Hoffmann by a human-derived single chain antibody fragment. J Biol Chem. 2011; 286: 20892-20900.

182. Rodríguez-Rodríguez ER, Ledezma-Candanoza LM, Contreras-Ferrat LG, Olamendi-Portugal T, Possani LD, Becerril B, Riaño-Umbarila L. A single mutation in framework 2 of the heavy variable domain improves the properties of diabody and a related single-chain antibody. J Mol Biol. 2012; 423: 337-350.