The venom produced by different classes of arthropods and uses it as a biological control agent

Kabir Eyidozehi[1], **Sultan Ravan**[2]

[1]Ph.D. student of Agricultural Entomology, University of Zabol, Iran

[2] Associate Professor Plant Protection Department, University of Zabol, Iran

(Corresponding Author: Kabir Eyidozehi)

Abstract

Animal kingdom possesses numerous poisonous species that produce venoms or toxins. The biodiversity of venoms and toxins made it a unique source of leads and structural templates from which new therapeutic agents may be developed. Such richness can be useful to biotechnology and/or pharmacology in many ways, with the prospection of new toxins in this field. Venoms of several animal species such as snakes, scorpions, toads, frogs and their active components have shown potential biotechnological applications. Recently, using molecular biology techniques and advanced methods of fractionation, researchers have obtained different native and/or recombinant toxins and enough material to afford deeper insight into the molecular action of these toxins. Now a day to visualize the boundaries between cancerous tissues and normal tissues florescent labeled scorpion venom peptides are used. Still a lot of peptides in scorpion venom are not identified. Further studies are needed to identify therapeutically crucial peptides in scorpion venom. This paper reviews the knowledge about the various aspects related to the name, biological and medical importance of poisonous animals of different major animal phyla.

Key words: Poisonous animals, Scorpion, Spider, Venoms, Insects

INTRODUCTION

1. The biological and medical significance of poisonous animals

Animal venoms and toxins are now recognized as major sources of bioactive molecules that may be tomorrow's new drug leads. Venom is a secretion produced in a specialized gland in one animal and delivered to a target animal through the infliction of a wound. This secretion must contain molecules that disrupt normal physiological processes (Fry *et al*., 2006). Venoms may be used to kill prey and/or to defend the delivering organism against attack by predators. Venoms are complex mixtures of pharmacologically highly active substances and can cause a wide range of symptoms (Junghanss and Bodio, 2006). The venom which contains mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamine, enzyme inhibitors, and protein is namely neurotoxic peptide (Table 1) (Müller, 1993; Gwee *et al*., 1994). The neurotoxic peptides are responsible for the symptoms that present during envenomation by interacting with ion channels and have the potential to cause massive damage to nervous system of both vertebrates and invertebrates (Mebs, 2002). Pre-synaptic neuromuscular junction neurotoxins act at the neuromuscular junction, damaging the terminal axon followed by cessation of all neurotransmitter release and irreversible paralysis. Post-synaptic toxins act by reversible binding to the acetylcholine receptor on the skeletal muscle end plate. Tetrodotoxin found in saliva of the Australian blue ringed octopus and the flesh of puffer fish causes rapid and reversible paralysis of skeletal muscle by blocking nerve transmission through action on the sodium channels of axons. A variety of potassium channel blocking toxins exist in the venoms of some scorpion and cone shell. Some snake venoms contain myolysins which caused myolysis of skeletal muscles, however, cardiac effects are prominent in envenoming by scorpions, jellyfish and cone shells. Some venoms contain true anticoagulants components that directly inhibit portions of the clotting cascade resulting in prolonged clotting times (Table 2). The viperid zinc metalloproteinases causes capillary leakage resulting in haemorrhagic necrosis. Renal damage may follow envenoming by a wide range of venomous animals as a secondary effect of venom induced hypotension. Some snakes e.g vipers, pit vipers, cobras commonly cause major local tissue injury, as a result of cytolytic phospholipase A2 toxins. A few spiders cause local necrosis as the

most prominent feature of envenomnation (Meier and White, 1 995; Williamson *et al*., 1996). Venom allergens cause immunostimulation of body tissues and show strong T cell responses in hypersensitive patients and signify the production of allergen specific IgE antibodies and generate anaphylactic reactions. Generally, venom toxins make fast release of certain chemicals serotonins, kinins, prostaglandins and leukotrienes that results in visible clinical symptoms related to paralysis, inflammation, swelling and itching (Upadhyay and Ahmad, 2010).

Envenomation of humans by scorpion stings constitutes a serious health problem in certain regions of the word (Fry *et al*., 2006). The most important components, responsible for severe intoxication are short- and long-chain peptides that affect ion-channel (Na+, K+, Ca+2, Cl-) function, either by blocking the channels or modifying their gating mechanisms (Junghanss *et al*., 2006). The best known are those specific for Na+ and K+ channels (Müller, 1993; Gwee *et al*., 1994). They cause abnormal depolarization of the cells and if not treated on time can lead to death. However many components might be present: enzymes such as phospholipase A2, proteases, hyaluronidase and other peptides with bradykinin-potentiating, antimicrobial, hemolytic and immune-modulating activities (Mebs, 2002). Anti-venoms have been prepared by hyper-immunization of horses, and their immunoglobulins have been purified and are currently used for control of envenomation (Espino-Solis *et al*., 2009).

However, due to rich variety of components present in these venoms there are some that have shown potential applications as therapeutic agents. The advancements in biotechnology have made it possible to synthesize new natural products such as components of venom purified with therapeutic properties. The therapeutic effects of these agents are usually achieved by mechanisms that are different from that of conventional therapeutic agents. Scorpion and its organs have been used to cure epilepsy, rheumatism and male impotency since medieval times. This review is focus at certain scorpion venom components and their potential applications for the treatment of various diseases including autoimmune, cardiovascular, infectious, inflammatory, hematological and malignant.

Snake venoms have attracted much medical attention since ancient civilizations and had been used in medical treatment for thousands of years. In the 12th century, doctors used snake venom to treat leprosy. Snakes belong to the order Squamata and suborder Serpentes (Ophidia) of the class Reptilia (Kochva, 1987). They appeared in the Lower Cretaceous Period about 130 million

years ago, and have been considered to have evolved from lizards, Varanidae, probably 30 million years ago (Clifford, 1955). There are about 3200 species of snakes found worldwide, and 1300 of which are venomous (Hider, 1991). Venomous snakes belong to the infraorder Caenophidia under Ophidia. They are more advanced and widespread throughout the world (Phelps, 1989). Venomous snakes usually are defined as those possessing a pair of venom glands and specialized fangs connected to venom glands by ducts. The venom apparatus enables them to inflict serious bites in their victims. The venom apparatus of a snake typically consists of a venom gland, venom duct and one or more fangs located on each side of the head . Venom is produced in paired modified salivary glands which in most venomous snakes are located superficially beneath the scales in the posterior part of the head and eyes. The gland is linked to the fang by a duct. Contraction of muscles around the gland compresses the gland, forcing the flow of venom along the duct to the fang where the size of these structures depends on the size and species of the snakes. Generally, four families of venomous snakes are known: *Elapidae* (cobra, mamba); *Hydrophiidae* (sea snakes); *Viperidae* (true vipers and pit vipers and rattlesnakes) and *Colubrida*e (Fenton, 2002).

The elapids are a large group of venomous snakes, which are distributed over Africa, Asia, the Southern parts of North America, Central and South America and Australia (Phelps, 1989). There are about two hundred and twenty species of elapid snakes which are represented by sixty-two genera. Generally, the elapids possess fixed front fangs that are situated in the front of the upper jaw. Most elapids are either terrestrial or aquatic; only two genera are arboreal, the mambas and the tree cobras, *Pseudohaje* (Phelps, 1989).

Venoms are the secretion of venomous animals, which are synthesized and stored in specific areas of their body venom glands. The animals use venoms for defense and/or to immobilize their prey. Most of the venoms are complex mixture of biologically active compounds of different chemical nature such as multidomain proteins, peptides, enzymes, nucleotides, lipids, biogenic amines and other unknown substances. Venomous animals as snakes, spiders, scorpions, caterpillars, bees, insects, wasps, centipedes, ants, toads and frogs have largely shown biotechnological or pharmacological applications. During long-term evolution, venom composition underwent continuous improvement and adjustment for efficient functioning in the killing or paralyzing of prey and/or as a defense against aggressors or predators. Different venom

components act synergistically, thus providing efficiency of action of the components. Venom composition is highly species-specific and depends on many factors including age, sex, nutrition and different geographic regions. Toxins, occurring in venoms and poisons of venomous animals, are chemically pure toxic molecules with more or less specific actions on biological systems (Gomes *et al*., 2010- Kang *et al*., 2011). A large number of toxins have been isolated and characterized from snake venoms and snake venoms repertoire typically contain from 30 to over 100 protein toxins. Some of these molecules present enzymatic activities, whereas several others are non-enzymatic proteins and polypeptides. The most frequent enzymes in snake venoms are phospholipases A2, serine proteinases, metalloproteinases, acetylcholinesterases, L-amino acid oxidases, nucleotidases and hyaluronidases. Higher catalytic efficiency, heat stability and resistance to proteolysis as well as abundance of snake venom enzymes provide them attractive models for biotechnologists, pharmacologists and biochemists (Kang et al., 2011- Murayama *et al*., 1997). Scorpion toxins are classified according to their structure, mode of action, and binding site on different channels or channel subtypes. The venom is constituted by mucopolysaccharides, hyaluronidases, phospholipases, serotonins, histamines, enzyme inhibitors, antimicrobials and proteins namely neurotoxic peptides. Scorpion peptides presents specificity and high affinity and have been used as pharmacological tools to characterize various receptor proteins involved in normal ion channel functionating, as abnormal channel functionating in cases of diseases. The venoms can be characterized by identification of peptide toxins analysis of the structure of the toxins and also have proven to be among the most and selective antagonists available for voltage-gated channels permeable to K+, Na+, and Ca2+. The neurotoxic peptides and small proteins lead to dysfunction and provoke pathophysiological actions, such as membrane destabilization, blocking of the central, and peripheral nervous systems or alteration of smooth or skeletal muscle activity (Quintero-Hernández *et al*., 2011; Petricevich, 2010).

Spider venoms are complex mixtures of biologically active compounds of different chemical nature, from salts to peptides and proteins. Specificity of action of some spider toxins is unique along with high toxicity for insects, they can be absolutely harmless for members of other taxons, and this could be essential for investigation of insecticides. Several spider toxins have been identified and characterized biochemically. These include mainly ribonucleotide phosphohydrolase, hyaluronidases, serine proteases, metalloproteases, insecticidal peptides and

phospholipases D (Tambourgi *et al*., 2004- Senff-Ribeiro *et al*., 2008). Venoms from toads and frogs have been extensively isolated and characterized showing molecules endowed with antimicrobial and/or cytotoxic activities (Habermehl, 1995). Studies involving the molecular repertoire of the venom of bees and wasps have revealed the partial isolation, characterization and biological activity assays of histamines, dopamines, kinins, phospholipases and hyaluronidases. The venom of caterpillars has been partially characterized and contains mainly ester hydrolases, phospholipases and proteases (Habermann, 1972). The purpose of this chapter is to present the main toxins isolated and characterized from the venom of venomous animals, focusing on their biotechnological and pharmacological applications.

2. Phylum Arthropoda

Basically according to Koehler and Diclaro (Koehler and Diclaro, 201 2) venomous arthropods produce venoms that can be classified as; venoms that produce blisters (blister beetles, certain stinging caterpillars, millipedes), venoms that attack the central nervous system (black and brown widow spiders, bark scorpions, certain ticks, Hymenoptera, wheel bugs), venoms that destroy tissue, or cytolytic and hemolytic toxins (Hymenoptera, fire ants, ground scorpions, mites, chiggers, wheel bugs, brown recluse spider), venoms that prevent blood from clotting, or hemorrhagic toxins (lice, fleas, ticks, mites, true bugs, biting flies).

2. 1. Class Insects

Venomous insects are known from the orders Lepidoptera, Hemiptera, and Hymenoptera (Blum, 1981). The method of delivery may be active, such as the sting apparatus of Hymenoptera (bees and wasps), and the mouthparts of Hemiptera (stylets), or passive such as the modified setae in some lepidopteran larvae (caterpillars). Hymenopterans are insects that inject venom with a stinging apparatus connected to venom glands in the terminal part of the abdomen. Some species of ants lack a sting and instead spray their venom. Honeybees and wasps are widely and numerously distributed in cold and tropical climates; therefore, most humans experience multiple stings during a lifetime. Single stings are dangerous for people who are allergic to the venom. Direct toxic effects, as opposed to allergic reactions account for 1 5% of all deaths caused by hymenopteran stings (Müller, 1990). Insect venom is a poisonous substance that contains a complex mixture of certain proteins, enzymes, small peptides, certain inorganic elements and

acids. These venom components are responsible for multiple pharmacological effects in different organisms. These venoms act at cellular level and break the normal barrier to leak out molecules across the cell membrane and form ion channels by attaching themselves to the membrane surface. Insect venom toxins elevate the level of blood sugar, lactate, glucagon and cortisol and cause massive destruction of erythrocytes and nerve cells. In addition, insect venom possess highly potent short peptides act on ion channels of excitable cells and inhibit the activity of important metabolic enzymes. Melittin is a short peptide that shows cytotoxicity and cause intravascular hemolysis of erythrocytes, leucocytes, platelets and vascular endothelium. It is highly basic peptide that inserts itself into the phospholipid bi-layer of cell membranes (Upadhyay and Ahmad, 2010). Venom secreted from the salivary glands of ticks during the blood meal is absorbed by the host and systemically distributed. Paralysis results from the ixovotoxin, very similar to botulinum toxin due to inhibition of the acetylcholine release at the neuromuscular junction and autonomic ganglia (Murnaghan,1960; Grattan *et al*., 1997).

Both ixovotoxin and botulinum toxin demonstrate temperature dependence in rat models and shows increased muscular twitching activity as the temperature is reduced (Cooper *et al*., 1976). The antimicrobial venom peptides of honey bee *Apis mellifera* are present on the cuticle of adult bees and on the nest wax. I t has been suggested that these substances act as a social antiseptic device. Venom functions are well beyond the classical stereotype of defense against predators, and the different nesting biology of these species may be related to the use of the venom in a social immunity context (Baracchi *et al*., 2011). Bumblebee venom contains a variety of components, including bombolitin, phospholipase A2, serine proteases, and serine protease inhibitors. A bumblebee (*Bombus terrestris*) venom serine protease inhibitor that acts as a plasmin inhibitor was consists of a 58- amino acid mature peptide that displays features consistent with snake venom Kunitz-type inhibitors, including six conserved cysteine residues and a P1 site (Qiu *et al*., 2013). *Solenopsis* fire ants are native to the Americas, with most of the species occurring in lower regions of South America (Tschinkel, 2013). Fire ant venom includes more than 95% piperidinic alkaloids and less than 5% aqueous fraction of allergenic proteins (Hoffman *et al*., 1990; Hoffman *et al*., 2005).

2. 2. Class Arachnida

From the venom of arachnids (scorpion and spiders) several hundred peptides have been isolated and characterized, most of which are relatively short peptides that interfere with cellular communication and impair proper function. Cloning genes extracted from the venomous glands of arachnids is revealed thousands of novel sequences (Quintero *et al*., 2011). Spiders employ venom jaws that are connected to venom glands to catch prey and for use in self defense. Most spiders either have venom jaws that are too small to penetrate human skin or their venom is too weak to produce substantial envenoming. Spider bites may go unnoticed until clinical signs and symptoms develop. Systemic neurotoxic envenoming is caused by widow spiders (*Latrodectus* sp.), wandering spiders (*Phoneutria* sp.), and funnel web spiders (*Atrax* sp. and *Hadronyche* sp.) are resembles envenoming from scorpion stings. The clinical course of envenoming by these spiders is also predominantly triggered by catecholamine release (Müller,1993; Sutherland and Tibballs, 2001; Warrell, 2003). Over a period of more than 300 million years, spiders have evolved an extensive library of bioactive peptides. Moreover, in contrast with man-made combinatorial peptide libraries, spider-venom peptides have been preoptimized for high affinity and selectivity against a diverse range of molecular targets. I t is therefore not surprising that numerous spider-venom peptides have been characterized that potently and selectively modulate the activity of a diverse range of therapeutic targets (Saez *et al*., 2010).

Spider venoms are complex mixtures of neurotoxic peptides, proteins and low molecular mass organic molecules. Their neurotoxic activity is due to the interaction of the venom components with cellular receptors in particular ion channels. Spider venoms have proven to be a rich source of highly specific peptide ligands for selected subtypes of potassium, sodium and calcium channels, and these toxins have been used to elucidate the structure and physiological roles of the channels in excitable and non-excitable cells (Escoubas *et al*., 2000 ; Hogan *et al*., 2004). The Brazilian tarantula *Acanthoscurria paulensis* venom induced many behavioral and physiological changes in mice. An inotropic effect produced on frog heart is probably due to the low molecular mass compounds present in the more hydrophilic fractions of venom that may act either by inducting the release of acetylcholine from parasympathetic terminals or by directly acting as a cholinergic agonist (Frangež *et al*., 2012).

Loxoscelism is a set of signs and symptoms caused by the bite of spiders of the genus *Loxosceles* (Da Silva *et al*., 2004). *Loxosceles* (Araneae, Sicariidae) can be found in temperate and tropical

regions of America, Oceania, Asia, Africa and Europe (Swanson *et al*., 2006; Souza *et al*., 2008). This genus represents a public health problem in Brazil, mainly in South and Southeast regions, with more than 3000 cases reported annually by the Ministry of Health (Hogan *et al*., 2004). Usually, the clinical manifestations of loxoscelism are characterized by necroulcerative dermatitis at the site of the bite. However the envenoming can also cause systemic effects leading to acute renal failure, which may be lethal (Hogan *et al*., 2004; Málaque *et al*., 2002; Abdulkader *et al*., 2008). Locally, lesions caused by loxosceles venom present edema, hemorrhage, inflammation with dominance of neutrophils, rhabdomyolisis, damage to the vessels wall, thrombosis, and dermonecrosis (Ospedal *et al*., 2002, Pereira *et al*., 2010). Recently, by using a cDNA library and transcriptome analysis, a novel expression profile has been elaborated for *Loxosceles intermedia* gland venom. This recently developed profile has allowed the identification of additional toxins as components of the venom, including insecticidal peptides similar to knottins, astacin-like metalloproteases, venom allergen, a translationally controlled tumor protein family member, serine protease inhibitors, and neurotoxins similar to Magi 3 (Corzo *et al*., 2003 , Gremski *et al*., 2010). In addition, the biotechnological use of *Loxosceles* toxins could provide information related to the tridimensional structure of identified toxins, through crystallography and X-ray diffraction and/or nuclear magnetic resonance for soluble toxins (Biley and White, 2001), from such data, synthetic ligands, analogs, or inhibitors could be designed for biotechnological purposes (Chaim *et al*., 2011).

Scorpions are found in all the world causes problems in tropical and subtropical regions. Travelers are stung when they accidentally squeeze scorpions that are hiding in beds, luggage, shoes, and clothing (Keegan, 1980). Scorpions are actually very beneficial to ecosystems because they eat insects, spiders, centipedes and even other scorpions. In turn, they provide an important food source for large centipedes, tarantulas, snakes, some lizards, birds, bats, and other small mammals. Scorpion venom varies from species to species, but generally consists of different mixtures of neurotoxins) Dolan and Mannan, 2009). Scorpion toxins are classified according to their structure, mode of action, and binding site on different channels or channel subtypes (Possani *et al*., 2000). The long chain toxins affecting sodium channels have been subdivided primarily into two major subtypes, and toxins)Jover *et al*., 1980). The ^-toxins bind to receptor

site 3 of the voltage-gated Na+ channels of vertebrates in a membrane-dependent manner (Catterall, 1992). The major effects of toxins induce a prolongation
of the action potential of nerves and muscles by fast inactivation of sodium channels receptor affinity dependent upon membrane potential (Possani *et al*., 2000, Possani *et al*., 1999). The `-toxins are isolated from American scorpions, bind to receptor site 4 on vertebrate Na+ channels and producing a shift to amore negative membrane potential (Cestele and Catterall, 2000; Shichor *et al*., 2002). So, Scorpion `-toxins have been used as pharmacological tools in the study of voltage-activated Na+ channels (Escoubas *et al*., 2000).

Systemic envenoming is caused by members of the genera *Centruroides*; *Tityus*; *Androctonus*, *Buthus*, *Leiurus*, *Nebo*; *Hemiscorpius*; *Parabuthus*; and *Mesobuthus* (Junghanss and Bodio, 2006). Local envenoming causes pain, erythema, and swelling. Systemic envenoming usually develops in two phases: a cholinergic phase involving vomiting, sweating, hypersalivation, priapism, bradycardia, and arterial hypotension, followed by an adrenergic phase involving arterial hypertension, tachycardia, and cardiac failure. Cranial nerves and neuromuscular junctions and respiratory organs may be affected (Curry *et al*., 1983). The mediators affecting inflammatory processes may be released after scorpion envenomation including kinins, ecosanoids, platelet activating factor, nitric oxide, and cytokines (Petricevich, 2010). A total of 74 fractions were separated from the Urodacidae scorpions, the most widely distributed in Australia, allowing the identification of approximately 274 different molecular masses with molecular weights varying from 287 to 43.437 Da. The most abundant peptides were those from 1 kDa and 4–5 kDa representing antimicrobial peptides and putative potassium channel toxins, respectively. The transcriptome analysis of the venom glands of the same scorpion species, resulting cDNA library 1 72 expressed sequence tags (Luna *et al*., 2012).

3. Phylum Molusca

The most important venomous mollusks are from the Gastropoda and Cephalopoda classes.

Gastropoda (genus *Conus*) contain mollusks able to envenom prey and occasionally even Man. Octopus vulgaris is a common marine animal that can be found in nearly all tropical and semitropical waters around the world. *Octopus vulgaris* bite resulting in an ulcerative lesion with slow wound healing owing to *P. oryzihabitans* infection (Aigner *et al*., 2011). The blueringed octopus (*Hapalochlaena maculata* and *Hapalochlaena lunulata*) inoculates maculotoxin from

their saliva glands through their horny beak. Recently maculotoxin was demonstrated as identical to tetrodotoxin (Sheumack *et al*., 1978). Tetrodotoxin is a potent neurotoxin that blocks axonal sodium channels and provokes a muscular paralysis similar to that observed in accidents with *Conus* shells including fatal respiratory arrest (Walker, 1983). Mollusks of the genus *Conus* present a venomous apparatus composed of radulae, a chitin structure linked to glands, which injects potent neurotoxic peptides, conotoxins, causing serious human envenomation that is associate with the blockage of certain receptors and muscular paralysis (Junior *et al*., 2006). The venom from any one *Conus* species contains a large number of peptides. Every conotoxin serves as a highly specific ligand, each with a particular molecular target. Binding of the peptide ligand to its target leads to a biologically relevant change in physiological function (Olivera and Cruz, 2001). Currently, conotoxins are a valuable tool of scientific research due to the intense pharmacological activity presented by the peptides. One of the drugs in clinical tests is ziconotide which is a peptide that blocks the neuronal calcium canals with excellent effect in the treatment of chronic and severe painful processes (Miljanich and Ziconotide, 2004). Moreover, conotoxins from different superfamilies were commonly found to have similar distributions. A new conotoxin, PCCSKLHDNSCCGL was sequenced (James, 2010). Conotoxins composed mostly of 1 00–250 disulfide-bridged peptides are synthesized in the epithelial cells of the venom duct, then secreted in discrete parts of the same duct, a convoluted gland often several centimeters long (Tayo *et al*., 2010). Conotoxins bind to receptors such as voltageand ligand-gated ion channels, G-protein-coupled receptors, and neurotransmitter transporters in the muscular and nervous system (Moller and Mari, 2001).

Conotoxins provide a vast library of peptides with unique abilities to discriminate among types and subtypes of ion channels in a manner that is unmatched by the typical small molecule drugs which dominate the pharmaceutical industry. In addition, cone venom peptides are small and inherently stable, making them ideal leads for peptide therapeutics, especially ion channel therapeutics. The high structural resolution now obtained with modern NMR spectroscopy and X-ray crystallography provides emerging opportunities to use conotoxins as templates for the design of smaller peptidomimetics that incorporate the selectivity and potency of conotoxins. Because of its selectivity and potency, w-conotoxin MVI IA (Ziconotide) is being developed as a

drug for the treatment of chronic pain. With improvement in methods of delivering peptides, it is anticipated that conopeptides can be modified for effective oral delivery (Adamsa *et al*., 1999).

4. Venom toxins

4.1. Scorpions venoms

Scorpion body is divided into three parts: the head (cephalothorax), the abdomen (mesosoma) and the tail (metasoma). Scorpions are venomous arthropods, members of *Arachnida* class and order *Scorpiones*. These animals are found in all continents except Antarctica, and are known to cause problems in tropical and subtropical regions. The scorpion species that present medically importance belonging to the family *Buthidae* are represented by the genera *Androctonus, Buthus, Mesobuthus, Buthotus, Parabuthus* and *Leiurus* located in North Africa, Asia, the Middle East and India. *Centruroides spp* are located in Southwest of United States, Mexico and Central America, while *Tityus spp* are found in Central and South America and Caribbean. In these different regions of the world the scorpionism is considered a public heath problem, with frequent statements that scorpion stings are dangerous. The signs of the scorpion envenomation are determined by the: a) scorpion species; b) venom composition and c) the victim´s physiological reaction to the venom. The symptoms start immediately with a few minutes after the sting and usually progress to a maximum severity within 5 hours. At this period the massive release of neurotransmitters results in sweating, nausea and vomiting (Ismail,1995; Mebs, 2002). The victims may exhibit signs and symptoms involving the central nervous system, stimulation of the autonomic nervous system, and occasionally, respiratory and heart failure, and even death. The victims of scorpion envenoming that presented multi-system-organ failure characterized by changes in hormonal environment with a massive release of counter-regulatory hormones, such as catecholamine, glucagon, cortisol, angiotensin-II, and with decreased levels of insulin and an increase blood glucose level. The grading of these scorpions envenomation depend local signs and whether or not neurological signs predominate. The local signs observed in victims can present effects that can separate in a neurotoxic and cytotoxic local. Central nervous system signs are: sympathetic, parasympathetic, somatic, cranial and peripheral nervous system. The signs are also classified as non-neurological (cardiovascular, respiratory, gastrointestinal, genitourinary, hematological, and metabolic signs), and neurological signs (release of catecholamine from the

adrenal glands or the release of acetylcholine from postganglionic parasympathetic neurons) (Freire *et al*., 1974).

Scorpions use their venoms for killing or paralyzing their prey. The venom helps the capture and digestion of praise, but also can serve to defend them against predators. The venom is constituted by mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamine, enzyme inhibitors and proteins usually named neurotoxins (Müller, 1993; Gwee *et al*., 2002). This reflects millions of years of evolution of specialized venom producing glands. Scorpions are among the oldest (400 million years) living groups of animals. They are represented by 1,500 distinct species and sub-species and their venoms are a mixture of components containing about 50 – 100 distinct polypeptides (Dehesa-Dàvila et *al*., 1994; Lehmann and Jurkat, 1999).

Potassium channels toxins (KTx) play an important role in a large variety of biological processes and their therapeutic value are involved in an increasing number of human pathologies specially autoimmune disorders, inflammatory neuropathies and cancer (Ashcroft and Gribble, 2000, Shieh *et al*., 2000). The scorpion toxin that target K+ channels (KTx) are composed by circa 31-39 amino acid residues. The potassium channels specific toxins are authentic blockers of the channels; they bind to the extracellular face of the channel and impede the flow of ions through the biological membrane. The -KTx family is constitutes by more than 50 different -KTx. They have been reported and listed in more than 18 families (Tytgat *et al*., 1999; Corona et al., 2002; Tenenholz *et al*., 2000). Various studies describe the three-dimensional structure of these KTx toxins. In case of *T. serrulatus* venom the neurotoxin -KTx 12.1 initially named as TsTX-IV is constituted by four disulfide-bridged (Tytgat *et al*., 1999; Batista *et al*., 2002; Arantes et al., 1989; Pimenta *et al*., 2003). The voltage gated potassium channel has been shown to play a role in decreasing of T cell activation and delayed type hypersensitivity (Villalonga *et al*., 2007). In venoms of three Brazilian scorpions *T. serrulatus, T. bahiensis* and *T. stigmurus*, the butantoxin has shown to block reversibly the potassium channels and inhibit the proliferation of T cells and IL-2 production (Holaday *et al*., 2000).

5. Therapeutic use of scorpion venom autoimmune diseases

Immunoregulatory abnormalities have been shown to exist in a wide variety of autoimmune and chronic inflammatory diseases including systemic lupus erythematosis, chronic rheumatoid arthritis, diabetes mellitus types I and II, inflammatory bowel disease, cirrhosis biliar, uveitis,

multiple sclerosis and other disorders such as Crohn´s disease, ulcerative colitis, psoriasis, ichthyosis and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different they have in common the appearance of a variety of autoantibodies and self-reactive lymphocytes. Such self-reactivity may be due, in part to a loss of the homeostatic controls under which the normal immune system operates. The end result of an autoimmune or a rejection process is tissue destruction caused by inflammatory cells and the mediators they release. Anti-inflammatory agents act principally by blocking the effect or secretion of these mediators without modifying the immunologic basis of the disease. The process of inhibition of potassium channels has been described by immunosuppressive response. Potassium channels can modulate a number of cellular events such as muscle contraction, neuro-endocrine secretion, frequency and duration of action potentials, electrolyte homeostasis, and resting membrane potential. The process of inhibition of potassium channels has been described by immunosuppressive response. Scorpion venoms have been recognized as a source of peptidyl inhibitors of various types of potassium-channels. Some of these peptides are capable of depolarizing human T cells, and preventing inflammatory and proliferative responses, and thus might play a potent treatment of autoimmune diseases, in the prevention of rejection of foreign organ transplants and/or related afflictions diseases and illness. The recently described Vm23 and Vm24 are capable of decreasing significantly the delay type of hypersensitive (DTH) in rats, applied at very low amounts (10 micrograms per rat), (Varga *et al*., 2012). In Table 1 are described the peptides with potential for the treatment to autoimmune diseases.

6. Antivenom production

Scorpion antivenom treatment, initially introduced in 1909, is still the only method used for the therapy against scorpion stings (Balozet, 1971; Theakston *et al*., 2003). The first application of the venom of scorpions is the preparation of heterologous antibodies capable of been used as anti-venoms. Normally, homogenates of telsons are used to prepare a raw extract that is injected in small dosis to horses and/or sheeps with increasing amounts during several months (Tulga, 1964). After a long period of immunization, the blood of the hiper-immunized animal is obtained and the immunoglobulins are purified for use as anti-venoms. 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 (Espino *et al*., 2009). 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 (Holliger and Hudson, 2005). Single-chain Fvs are a 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 (Holliger and Hudson, 2005). In this context, recently single chain antibodies of human origin were developed and shown to be effective for neutralization of scorpion toxin envenomation (Riaño-Umbarila *et al*., 2011; Canul-Tec *et al*., 2011; Rodríguez-Rodríguez *et al*., 2012)

6.1.Cardiac diseases

Cardiac diseases are constituted by coronary heart and cerebro-vascular diseases. Peptides from animal venoms are active as bradykinin-potentiating factors are of particular interest because of their strong effect as hypotensive agent. These factors have been found in *Leiurus quinquestriatus, Tityus serrulatus, Buthus martensii* and *B. occitanus* scorpions. Pharmacologically, these peptides obtained from scorpions venoms act as bradykinin-potentiating peptides and can be used as hypotensive agents in the treatment of hypertension. Moraes *et al*., 2011 (Moraes *et al*., 2009) described that sodium channel gating from *Tityus bahiensis* scorpion venom modified present different effects on sodium channel isoforms.

6.2.Hematological diseases

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 was described to be present in the venom from the scorpion *Buthus martensii* Karsch (Song *et al*., 2005). This same peptide is related to the resistance against platelet aggregation and causes increment of the concentration of prostanglandin I2 in plasma (Song *et al*., 2005). *Tityus discrepans* scorpion venom modifies clotting times in humans. Brazon *et al*., 2008 (Brazón *et al*., 2009) described the effect of *T. discrepans* venom on a partial thromboplastin time prothrombin

time and its direct clotting activity. This venom contains anticoagulant components which prolong prothrombin time and partial thromboplastic time.

6.3. Infectious diseases

Cationic host defense peptides are produced by many organisms as part of their host defense system (Hancock and Sahl , 2006; Wang *et al*., 2006). These peptides are considered as antimicrobial agents against microorganisms such as: bacteria, fungi, parasites and virus (Cahalan, 1975; Brogden *et al*., 2003). Various studies are shown that the targets of cationic host defense peptides varied from the outer membrane to the signaling pathway (Brown and Hancock, 2006; Jenssen *et al*., 2006). These peptides are usually constituted of 10-50 amino acids (Hancock and Sahl, 2006). The diversity of scorpion venom is well known to contain about 400 such polypeptides with or without disulfide bonds. In the literature various studies described the presence of cationic host defense peptides in hemolymph and venoms from different species of scorpions.

The vaccination with SARS-CoV, influenza A (H5N1, H1N1) and measles virus have demonstrated variable efficacy. The cationic host defense peptides from scorpion venom can be modified for antiviral activity, especially against SARS-CoV, influenza A and measles virus. Another study described by Li *et al*., 2011, identified the microporin, a cationic host defense peptide from scorpion venom, which can effectively inhibit bacteria growth. The optimized microporin-M1 can inhibit grow of gram-positive bacteria at low concentrations and antibiotic-resistant pathogens.

6.4. Malignant diseases

Cancer is the major public health burden in all developed countries. The search for cancer cure from natural product such as plants and animals has been practiced for over a century and the use of purified chemicals to treat cancer still continues. With respect to chlorotoxin, it is considered a potent tool for early detection of skin, cervical, esophageal, colon and lung cancers (Liu *et al*., 2002). These ion-channels recognize by this toxin are among the many membrane proteins overexpressed in different types of cancers. Scorpion venoms have been used as traditional and folk therapy in various pathphysiological conditions that has been mentioned in folk and traditional medicine of India, China, Africa and Cuba (Liu *et al*., 2002). Various studies have

suggested that the cancer preventive and therapeutic efficacy of scorpion venom in different animal tumor models and cell culture systems might be usefull. Bioactivies polypeptides and enzymes as serine proteinase and hyaluronidase extract from scorpion venoms from different species has been exhibited as potential usefull as anti-proliferative agent with anti-tumor activity (Liu *et al*., 2002).

Reference

Fry BG, Vidal N, Norman JA, Vonk FJ, Scheib H, Ramjan SF, et al. Early evolution of the venom system in lizards and snakes. Nature. 2006; 439: 584-588.

Junghanss T, Bodio M. Medically important venomous animals: biology, prevention, first aid, and clinical management. Clin Infect Dis. 2006; 43(1 0): 1309-1 31 7.

Müller GJ. Black and brown widow spider bites in South Africa, a series of 45 cases. S Afr Med J. 1 993; 83: 399-405.

Gwee MCE, Gopalakrishnakone P, Yuen R, Khoo HE, Low KSY. A review of stonefish venoms and toxins. Pharmacol Therap. 1 994; 64(3): 509-528.

Mebs D. Scorpions and snakes, such as cobras, mambas and vipers made the African continent famous for venomous animals. Bull Soc Pathol Exot. 2002; 95 (3): 31 .

Meier J, White J. 1 995. Handbook of clinical toxicology of animal venoms and poisons. Boca Raton, FL, CRC Press.

Williamson JA, Fenner PJ, Burnett JW, Rifkin JF (eds.). 1996. Venomous and poisonous marine animals: a medical and biological handbook. Sydney: University of New South Wales Press.

Upadhyay RK, Ahmad S. Allergic and toxic responses of insect venom and it's immunotherapy. J Pharm Res. 201 0; 3(1 2): 31 23-31 28.

White J, Dart RC. 2007. Snakebite: A Brief Medical Guide. Stirling.

Rein KS, Borrone J. Polyketides from dinoflagellates: origins, pharmacology and biosynthesis. Comp Biochem Physiol B Biochem Mol Biol. 1 999; 1 24: 117-131 .

Isbister GK, John NA, Hooper JNA. Clinical effects of stings by sponges of the genus *Tedania* and a review of sponge stings worldwide. Toxicon. 2005; 46: 782- 785.

White J. 201 0. Venomous animals: clinical toxicology. Molecular, Clinical and Environmental Toxicology. Volume 2: Clinical Toxicology. pp: 233-291.

Fry BG, Vidal N, Norman JA, Vonk FJ, Scheib H, Ramjan SF, *et al*. Early evolution of the venom system in lizards and snakes. Nature. 2006; 439: 584-588.

Müller GJ. Black and brown widow spider bites in South Africa, a series of 45 cases. S Afr Med J. 1 993; 83: 399-405.

Sipkema D, Franssen MCR, Osinga R, Tramper J, Wijffels RH. Marine sponges as pharmacy. Marine Biotechnol. 2005; 7: 1 42-1 67.

Kitagawa I , Kobayashi M. Antitumor marine natural products. Gan To Kagaku Ryoho. 1 990; 1 7: 322-329.

Casapullo A, Bifulco G, Bruno I , Riccio R. New bisindole alkaloids of the topsentin and hamacanthin classes from the Mediterranean marine sponge *Rhaphisia lacazei*. J Nat Prod. 2000; 63: 447-451 .

Chen CY, Shen YC, Chen YJ. Bioactive sesquiterpenes from a Taiwanese marine sponge *Parahigginsia* sp. J Nat Prod. 1 999; 62: 573-576.

Russell FE. 1 965. Marine toxins and venomous and poisonous marine animals. In: Russell FS, ed. Advances in marine biology. Vol. 3. London, Academic Press.

Halstead BW. 1 988. Poisonous and venomous marine animals of the world. 2nd ed. New Jersey: Darwin Press.

Watson GM, Wood RL. 2008. Colloquium on terminology. In: The Biology of Nematocysts.

Belmonte G. , Menestrina G, Pederzolli C, Krizaj I , Gubensek F, Turk T, Macek P. Primary and secondary structure of a pore-forming toxin from the sea anemone, *Actinia equina* L. , and its association with lipid vesicles. Biochim. Biophys. Acta. 1 994; 1192: 1 97-204.

Diochot S, Baron A, Rash LD, Deval E, Escoubas P, Scarzello S, et al. A new sea anemone peptide, APETx2, inhibits ASIC3, a major acid-sensitive channel in sensory neurons. Embo J. 2004; 23: 1 51 6- 1525.

Bosmans F, Tytgat J. Sea anemone venom as a source of insecticidal peptides acting on voltage gated Na+ channels. Toxicon. 2007; 49: 550-560.

Turk T, William R, Kem WR. The phylum Cnidaria and investigations of its toxins and venoms until 1 990. Toxicon. 2009; 54: 1 031 -1 037.

Mariottini GL, Pane L. Mediterranean Jellyfish Venoms: A Review on Scyphomedusae. Mar. Drugs. 201 0; 8(4): 1 1 22-1 1 52.

Wang B, Zhang L, Zheng J, Wang O, Wang T, Lu J, et al. Multiple organ dysfunction: A delayed envenomation syndrome caused by tentacle extract from the jellyfish *Cyanea capillata*. Toxicon. 201 3; 61 : 54-61 .

Weinheimer AJ, Spraggins RL. The occurrence of two new prostaglandin derivatives (1 5-epi-PGA2 and its acetate, methyl ester) in the Gorgonian *Plaxaura homomalla*. chemistry of coelenterates. XV. Tetrahedron Lett. 1 989; 1 0: 51 85-51 88.

Moore RE, Scheuer PJ. Palytoxin: a new marine toxin from a Coelenterate. Science. 1971 ; 1 72: 495- 498.

Rouzaire-Dubois B, Dubois J. Characterization of palytoxin-induced channels in mouse neuroblastoma cells. Toxicon. 1 990; 28: 1 1 47-1 1 58.

Honda A, Yamamoto Y, Mori Y, Yamada Y, Kikuchi H. Antileukemic effect of coral-prostanoids clavulones from the stolonifer *Clavularia viridis* on human myeloid leukemia (HL-60) cells. Biochem Biophys Res Commun. 1 985; 1 30: 51 5-523.

Possani LD, Becerril B, Delepierre M, Tytgat J. (1999). Scorpion toxins specific for Na+channels. *Eur J Biochem.* Sep 264(2): 287-300.

Tytgat J, Chandy KG, Garcia ML, Gutman GA, Martin-Eauclaire MF, van der Walt JJ, Possani LD. (1999). A unified nomenclature for short-chain peptides isolated from scorpion venoms: alpha-KTx molecular subfamilies. *Trends Pharmacol Sci.* Nov 20(11): 444-7.

Espino-Solis GP, Riano-Umbarila L, Becerril B, Possani LD. (2009). Antidotes against venomous animals: state of the art and prospectives. *J Proteomics*. Mar 6;72(2):183-99. Epub 2009 Jan 23. Review.

Ismail M. (1995). The scorpion envenoming syndrome. *Toxicon*. Jul 33(7): 825-58.

Mebs D. (2002). Scorpions and snakes, such as cobras, mambas and vipers made the African continent famous for venomous animals. *Bull Soc Pathol Exot.* Aug 95(3): 131.

Freire-Maia L, Pinto GI, Franco I. (1974). Mechanism of the cardiovascular effects produced by purified scorpion toxin in the rat. *J Pharmacol Exp Ther.* Jan 188(1): 207-13.

Müller GJ. (1993). Scorpionism in South Africa. A report of 42 serious scorpion envenomations. *S Afr Med J.* Jun 83(6): 405-11.

Gwee MC, Nirthanan S, Khoo HE, Gopalakrishnakone P, Kini RM, Cheah LS. (2002). Autonomic effects of some scorpion venoms and toxins. *Clin Exp Pharmacol Physiol.* Sep 29(9): 795-801.

Dehesa-Dàvila M, Martin BM, Nobile M, Prestipino G, Possani LD. (1994). Isolation of a toxin from *Centruroides infamatus infamatus* Koch scorpion venom that modifies Na+ permeability on chick dorsal root ganglion cells. *Toxicon.* Dec 32(12): 1487-93.

Ashcroft FM, Gribble FM. (2000). Tissue-specific effects of sulfonylureas: lessons from studies of cloned K(ATP) channels. *J Diabetes Complications.* Jul-Aug 14(4): 192-6.

Lehmann-Horn F, Jurkat-Rott K. (1999). Voltage-gated ion channels and hereditary disease. *Physiol Rev.* Oct 79(4): 1317-72.

Possani LD, Merino E, Corona M, Bolivar F, Becerril B. (2000). Peptides and genes coding for scorpion toxins that affect ion-channels. *Biochimie.* Sep-Oct 82(9-10): 861-8.

Jover E, Couraud F, Rochat H. (1980). Two types of scorpion neurotoxins characterized by their binding to two separate receptor sites on rat brain synaptosomes. *Biochem Biophys Res Commun.* Aug 29; 95(4): 1607-14.

Wheeler KP, Barhanin J, Lazdunski M. (1982). Specific binding of toxin II from Centruroides suffusus suffusus to the sodium channel in electroplaque membranes. *Biochemistry.* Oct 26; 21(22): 5628-34.

Catterall WA. (1992). Cellular and molecular biology of voltage-gated sodium channels. *Physiol Rev.* Oct 72(4 Suppl): S15-48.

West JW, Patton DE, Scheuer T, Wang Y, Goldin AL, Catterall WA. (1992). A cluster of hydrophobic amino acid residues required for fast Na(+)-channel inactivation. *Proc Natl Acad Sci* U S A. Nov 15: 89(22): 10910-4.

Cestèle S, Catterall WA. (2000). Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. *Biochimie.* Sep-Oct 82(9-10): 883-92.

Cestèle S, Gordon D. (1998). Depolarization differentially affects allosteric modulation by neurotoxins of scorpion alpha-toxin binding on voltage-gated sodium channels. *J Neurochem.* Mar 70(3): 1217-26.

Gordon D. (1997). A new approach to insect-pest control-combination of neurotoxins interacting with voltage sensitive sodium channels to increase selectivity and specificity. *Invert Neurosci.* Sep-Dec 3(2-3): 103-16.

Marcotte P, Chen LQ, Kallen RG, Chahine M. (1997). Effects of *Tityus serrulatus* scorpion toxin gamma on voltage-gated Na+ channels. *Circ Res.* Mar 80(3): 363-9.

Cahalan MD. (1975). Modification of sodium channel gating in frog myelinated nerve fibres by *Centruroides sculpturatus* scorpion venom. *J Physiol.* Jan 244(2): 511-34.

Cestèle S, Yarov-Yarovoy V, Qu Y, Sampieri F, Scheuer T, Catterall WA. (2006). Structure and function of the voltage sensor of sodium channels probed by a beta- scorpion toxin. *J Biol Chem.* Jul 28: 281(30): 21332-44.

Cohen L, Karbat I, Gilles N, Ilan N, Benveniste M, Gordon D, Gurevitz M. (2005). Common features in the functional surface of scorpion beta-toxins and elements that confer specificity for insect and mammalian voltage-gated sodium channels. *J Biol Chem.* Feb 11: 280(6): 5045-53.

Armstrong CM, Bezanilla F. (1977). Inactivation of the sodium channel. II. Gating current experiments. *J Gen Physiol.* Nov: 70(5): 567-90.

Keynes RD. (1994). The kinetics of voltage-gated ion channels. *Q Rev Biophys.* Dec: 27(4): 339-434.

Yu FH, Catterall WA. (2003). Overview of the voltage-gated sodium channel family. *Genome Biol.* 4(3): 207.

Mouhat S, Jouirou B, Mosbah A, De Waard M, Sabatier JM. (2004). Diversity of folds in animal toxins acting on ion channels. *Biochem J.* Mar 15;378(Pt 3): 717- 26.

Bosmans F, Martin-Eauclaire MF, Tytgat J. (2007). Differential effects of five 'classical' scorpion beta-toxins on rNav1.2a and DmNav1 provide clues on species- selectivity. *Toxicol Appl Pharmacol.* Jan 1: 218(1): 45-51.

Shieh CC, Coghlan M, Sullivan JP, Gopalakrishnan M. (2000). Potassium channels: molecular defects, diseases, and therapeutic opportunities. *Pharmacol Rev.* Dec: 52(4): 557-94.

Corona M, Gurrola GB, Merino E, Cassulini RR, Valdez-Cruz NA, García B, Ramírez-Domínguez ME, Coronas FI, Zamudio FZ, Wanke E, Possani LD. (2002). A large number of novel Ergtoxin-like genes and ERG K+-channels blocking peptides from scorpions of the genus *Centruroides*. *FEBS Lett.* Dec 4: 532(1-2): 121-6.

Batista CV, Gómez-Lagunas F, Rodríguez de la Vega RC, Hajdu P, Panyi G, Gáspár R, Possani LD. (2002). Two novel toxins from the Amazonian scorpion *Tityus cambridgei* that block Kv1.3 and Shaker B K(+)-channels with distinctly different affinities. *Biochim Biophys Acta.* Dec 16: 1601(2): 123-31.

Tenenholz TC, Klenk KC, Matteson DR, Blaustein MP, Weber DJ. (2000). Structural determinants of scorpion toxin affinity: the charybdotoxin (alpha-KTX) family of K(+)-channel blocking peptides. *Rev Physiol Biochem Pharmacol.* 140: 135-85.

Arantes EC, Prado WA, Sampaio SV, Giglio JR. (1989). A simplified procedure for the fractionation of *Tityus serrulatus* venom: isolation and partial characterization of TsTX-IV, a new neurotoxin. *Toxicon.* 27(8): 907-16.

Holaday SK Jr, Martin BM, Fletcher PL Jr, Krishna NR. (2000). NMR solution structure of butantoxin. *Arch Biochem Biophys.* Jul 1: 379(1): 18-27.

Pimenta AM, Legros C, Almeida F de M, Manuselle P, De Lima ME, Bougis PE, Martin-Eauclaire MF. (2003). Novel structural class of four disulfide-bridged peptides from *Tityus serrulatus* venom. *Biochem Biophys Res Commun.* Feb 21: 301(4): 1086-92.

Villalonga N, Ferreres JC, Argilés JM, Condom E, Felipe A. (2007). Potassium channels are a new target field in anticancer drug design. *Recent Pat Anticancer Drug Discov.* Nov: 2(3): 212-223.

Varga,Z., Gurrola-Briones,G., Papp,F., Rodríguez de la Vega,R.C., Pedraza-Alva, G., Tajhya,R.B., Gaspar,R.,Cardenas,L., Rosenstein,Y., Beeton, C., Possani,L.D., Panyi,G. Vm24, a natural immunosuppressant peptide potently and selectively blocks Kv1.3 potassium channels of human T cells. *Molecular Pharmacology*, published ahead doi:10.1124/mol.112.078006, (2012).

Moerman L, Bosteels S, Noppe W, Willems J, Clynen E, Schoofs L, Thevissen K, Tytgat J, Van Eldere J, Van Der Walt J, Verdonck F. (2002). Antibacterial and antifungal properties of alpha-helical, cationic peptides in the venom of scorpions from southern Africa. *Eur J Biochem.* Oct; 269(19):4799-810.

Elgar D, Verdonck F, Grobler A, Fourie C, du Plessis J. (2005). Ion selectivity of scorpion toxin-induced pores in cardiac myocytes. *Peptides.* Jan;27(1):55-61. Epub 2005 Aug 8.

Ferreira LA, Henriques OB. (1992). Isolation of a bradykinin-potentiating factor from scorpion *Tityus serrulatus* venom. *Agents Actions* Suppl. ;38 (Pt 1):462-8

Ali AS, Stoeva S, Schutz J, Kayed R, Abassi A, Zaidi ZH, Voelter W. (1998). Purification and primary structure of low molecular mass peptides from scorpion (Buthus sindicus) venom. *Comp Biochem Physiol A Mol Integr Physiol.* Dec;121(4):323-32.

Balozet L. *Scorpionism in the Old World.* In: BÜCHERL W., BUCKLEY E. Eds. Venomous animals and their venoms. New York: Academic Express, 1971: 349-71.

Theakston RD, Warrell DA, Grifithis E. (2003). Report of a WHO workshop on the standardization and control of antivenoms. *Toxicon*. 41, 541-57.