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SPIDER VENOM TOXINS ITS BIOLOGICAL EFFECTS AND ALLERGIC-IMMUNE RESPONSES: A REVIEW

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ABSTRACT

Present review article elucidates various spider venom toxin types and their ion gated channel interactions and biological activities. Spider venom is a complex mixture of bioactive compounds such as enzymes, peptides, proteins, polyamines, salts and acids that show multiple biological effects in animals as well as in human. In present review recent information available on spider venom toxins i.e. Huwentoxin, Argitoxin: Guangxitoxin, Oxytoxin, Hainantoxin-1, Zinghaotoxin, Latrotoxins, Therophotoxin and their binding to a broad range of cellular targets with high affinity and selectivity, and remarkable structural diversity has been described in detail. Spider venom toxin peptides interact with ligand gated channels and modulate the activity of neuronal ion channels and receptors. These target voltage gated potassium K(V), calcium Ca(V) or sodium Na(V) channels. Spider-venom peptides target vertebrate or invertebrate Na(V) channels. They also target specific Na(V) channel subtypes that are considered to be important analgesic targets. No doubt spider venom peptides possess immense therapeutic potential against a wide range of pathophysiological conditions including cardiovascular disorders, chronic pain, inflammation and erectile dysfunction. However, after establishing interactions of these toxins with sodium channels could add the development of novel pain therapeutics. These can also use for development of more selective and potent hNaV1.7 inhibitors for treatment of chronic pain. By using molecular templates of spider toxins and using recent technologies spider toxins can be used for production of good therapeutic agents mainly antimicrobials, anticancer agents and bio-insecticides.

KEYWORDS: Spider toxins, voltage gates, biological activity, analgesics, anticancer, antimicrobials, bio-insecticides.

INTRODUCTION

Spiders are ancient predatory arachnids which possess highly toxic venom. According to an estimate there are more than 100000 spider species the worldwide. These were evolved from an Arachnid ancestor around 300 million years ago during the Carboniferous period. During long evolutionary timescale spider have evolved complex venom that is employed to subdue prey and deter predator animals.[1] Spider bites are a major problem in many Asian, African and other Tropical countries. The main purpose of production of venom in spider is to secure food and self-protection. They use venom for making effective defensive or device to deter and defend them by incapacitation. The vast majority of spiders employ a lethal cocktail to rapidly paralyze their prey, which are often many times their own size. However despite their fearsome reputation, less than a handful of these insects assessing are harmful to human. The predominant prey to spiders are smaller arthropods but larger species kill and feed on small fish, reptiles, amphibians, birds, mammals. Spiders are strong predatory animals which possess venom glands in their mouth apparatus that secrete toxins. These inflict the venom in the prey and immobilize it by inducing either respiratory paralysis or cardiac arrest and kill them. Spiders usually make a web thread to catch the insects, and inflict crude venom to paralyze them. Spider usually hunt insect suddenly from the side wall or hiding inside the crevices.

Nevertheless this is a small group of medically important species possesses remarkable pharmacological diversity of venom toxins which are extensive library of bioactive peptides. Thousands of bioactive substances and toxins have been isolated from spider venom most of them are low molecular weight peptides and proteins. Spider venom contains diverse toxins that target a range of receptors, channels and enzymes in wide range of vertebrates and invertebrate species. Spider venom includes substances of different chemical nature that can be conveniently divide in to three groups by molecular mass. These are low molecular weight compounds which are inorganic and organic in nature. Most of them are salts carbohydrates, amino acid, biogenic amines.[2] The second groups of compounds are peptides which are major venom compounds acylpolyamine and polypeptides found in millimolar concentration.[3] Spider are natural insect predator and use venom peptides as insecticides agents to paralyze the pray. Venom components possess function including induction of paralysis activation or inhibition of ion channels and receptors and microorganism killing. Spiders bear pharmacologically complex substances which cause rapid subjugation of prey. Most spider venom is dominated by disulphide rich peptides that typically have high affinity

and specificity for particular subtype of ion channels and receptors. Spider venom shows enough molecular diversity and approximately more than 10 million bioactive peptides whose templates are making them a valuable resource for drug discovery. Spider venom peptides possess immense therapeutic value against a wide range of pathophysiological conditions cardiovascular disorders, chronic pain, inflammation and erectile dysfunction. Spider venom toxin peptides interact with ligand gated channels, modulate the activity of neuronal ion channels and receptors. These target voltage gated potassium (Kv), calcium (Cav) or sodium (Nav) channels. Spider venom peptides bind on purinergic receptors, channels such as acid sensing ion channels, mechanosensitive channels and transient receptors potassium channels. Spider venom is concerned to be a potential source of modulators for all of drug targets. Spider venom toxins show action agent more than 65 blockers of vertebrates Cav channels and which of them found are active on Cav 2.2. Venom composition is highly species specific and depends on many climatic and biological factors. Spider venom contains a mixture of biologically active compounds with different biochemical activities. Spider venom includes substances of different chemical nature. Till the date more than 550 peptide and approximately 25% of polypeptides are of 10 kDa in molecular weight have been characterized from more than 60 spider species.

Structural attributes

Spider venom and its components

Spider venom comprises a mixture of compound with diverse biological activities, bind a broad range of cellular targets with high affinity and selectivity and appear remarkable structural diversity. Spider venom is a rich source of bioactive compounds and contains many enzymes, proteins, polyamines and acids which show multiple biological effects in animals as well as in human. High levels of chemical diversity make spider venoms attractive subjects for chemical prospecting. More than 500 bioactive peptides from venoms of about 60 spider species with molecular weight of less than 10 kDa are characterized and divided into 20 families.[4] Most of these spider peptides contain six or eight cysteine residues to form three to four disulfide bridges but they have different disulfide bond motifs. Most of these disulfide-containing peptides exhibit neurotoxic properties. Two large venom group are distinguished by character of their action-Neurotoxic and Necrotic (Cytolytic). Spiders produce venom with a predominance disulphide containing peptides of components. Phospholipase A2 is an important component of bee and snake venom[5] (Table 1).

Structural motifs in spider toxins

Spider toxin contains C1 x5-19 C2 G/Px2 C3X6-19C4 where X is any amino acids residues and arrangement of the di-sulphide bond is C1-C3, C2-C4. Different enzymes such as protease, nuclease, lipase, glycosidase etc are also identified in spider venom. Pure venom preparation shows very low proteolysis activity except from rare cases.[6] The venom of Loxosceles spider is a complex mixture of protein and peptides toxins with a molecular mass profile ranging from 1-40 kDa, venom including alkaline phosphatase, 5 ribonuleotide, phosphphydrolase, sulphate nucleosidase. Hyaleuronidase, Phospholipase, metalloprotease, serine protease and insecticide toxins. The diversity of protein component has been identified in venom of Chinese tarantulas *Chilobrachys jingzhoo* and *H. schmidti*, which are mainly rich with peptide components and revealed the presence of at least 90 and 300 proteins, respectively, with Mm >10 kDa. *Loxosceles laeta*) venom contains various enzymes.[7] An interesting peculiarity of genes of *H. schmidti* peptides and Latrotoxins is the absence of intron, which is unique, compared to other animal toxin genes, and is more characteristics of bacterial proteins. Spider venoms are complex cocktail compound of a variety of compounds, including salts, small organic molecule, peptides and proteins. It has been estimated that the potential number of unique spider venom peptides could be upwards of 12 million.[8]

Spider toxin types and subtypes

Huwentoxin-IVtoxin

Huwentoxin-IV (HWTX-IV) is found in venom of Chinese spider *Ornithoctonous huwena*.[9] It shows electrophysiological activity which is reversible and receptors retain its original bioactivity.[10] HWTX-XI toxins isolated from the spider venom glands of *Ornithovtnous huwena* are k unitz type toxins (KTTs) which are functionally voltage gated blockers.[11] The spider venom peptide 8-amaurobitoxin-PI1a, which targets snow drop lectin (GNA) and confer oral toxicity.[12] A novel 39 residue peptide, w-trtx-Cc1a (Cc1a), from the venom of the tarantula *Eitharisehius crawshyi* (noul pelinobius muticus) is isolated that shows 67% similarity to the spider toxin ω-TRTX-hg 1a.[13] CSTX-1 isolated from *Cupiennius salei* venom contain inhibitor cystine-knot motif that may act as a ion channel blocker. CSTX-1 produce a slow voltage-independent block of both mid/low- (M-LVA) and high-voltage-activated (HVA) insect Ca(v) channels. Since *C. salei* venom affects both insect as well as rodent species, CSTX-1 blocks rat neuronal L-type, but no other types of HVA Ca(v) channels, and failed to modulate LVA Ca(v) channel currents[14] (Table 1).

Native huwentoxin-IV and found that sodium channel isoforms from rat hippocampus neurons were also sensitive to native and synthetic toxins, but the toxin-binding affinity [IC(50) approximately 0.4 microM] was 12-fold lower than to peripheral is forms. The blokage by huwentoxin-IV could be reversed by strong depolarization due to the dissociation of toxin-channel complex as observed for receptor site 3 toxins. Moreover, small unilamellar vesicle-binding assays showed that in contrast to ProTx-II from the tarantula *Thrixopelma pruriens*, Huwentoxin-IV almost lakes the ability to partition into the negatively charged and neutral phospholipids bilayer of artificial membranes. These findings indicated that huwentoxin-IV was a sodium channel antagonist preferentially targeting peripheral is forms via a mechanism quite different from ProTx-II.[15]

Argitoxin

A natural argitoxin-636(Arg TX-636) and its two analogues Arg TX-75 & Arg TX-48 were isolated from venom of *Argiope lobata* spider.[16] These (agatoxins IV A& IV β) are usually used as diagnostic ligand to bind p-type Ca^{++} channels.[17] The peptidic toxin TX 3-1, a selective blocker of IA current, is extracted from the venom of the spider *phoneutria nigriventer*.[18] An astacin-like protease (LALP) was obtained from *loxosceles intermedia* venom. Spider *Oxyopes takobius* contain two domain modular toxin named spiderines: otTX1a, 1b & 2b which show insecticidal activity & protect antimicrobial effect.[4] Agatoxins are isolated from venom of funnel web spider *Agelenospsis aperta* that inhibits glutamate receptor prevent traction of insect muscle cells), μ-agatoxins (peptide neurotoxins, modulator activator of Na^{+} channel in number of insect neuronal cells which stimulate secretion of neuromediaters, a number of **ω**-agatoxin (peptide neurotoxins, inhibiter of Ca^{++} channel in neurons prevent release of neuromodulaters directly affecting various channel of insect and vertebrate (Table 1).

ω-conotoxin

ω -conotoxin MVIIA & pha 1β induce acute and chronic pain[19] while PhTx3-4, ω-Conotoxin MVIIA and MVIIC inhibit glutamate uptake and capsaicin-induced glutamate release and Ca^{++} ion spinal cord synaptosomes in calcium dependent manners. In contrast the Conos toxins, **ω** -conotoxin MVIIA and X-conotoxin MVIIC only inhibited calcium dependent glutamate release. PhTx 3-4 but not ω-conotoxin MVIIA or conotoxin MVIIC is able to inhibit the uptake of glutamate by synaptosomes and this inhibition in turn leads to a decrease in the Ca^{++} dependent release of glutamate. No another polypeptide toxin so far

decrease this effect. PhTx3-4 and ω conotoxin MVIIC and MVIIA are blocker of voltage dependent calcium channels, and they significantly inhibit the capsaicin-induced rise of intracellular Ca^{++}. In spinal cord, synaptosome, which likely reflect Ca^{++} ion entry through voltage gated Ca^{++} channels. Therefore, inhibition of calcium independent glutamate release by PhTx3-4 can be used to block glutamate release in pathological conduction such as pain (Table 1).

Guangxitoxin

Brown spider venom are complex mixture of toxins especially enriched in three molecular families, the phospholipase D, astacin like metalloproteases and inhibitor cysteine knot (ICK) peptides.[20] Kv2 family of spider toxins-guangxitoxin-1E block Kv2 is a selective blocker.[21] Yellow sac spider (*Cheiracanthium punctorium*) contains unique venom composition that possesses two domain chain punctorium toxins (CpTx) along with the corresponding cDNA and genomic DNA sequences.[22]

Oxytoxin

Oxytoxins (OxyTx1 and OxyTx2) are disulfide-rich peptides isolated from the venom of the spider *Oxyopes lineatus* that block voltage-sensitive calcium ion channels (VSCCs). OxyTx1 is identified in spider *Oxyopes kitabensis* but its pharmacological properties are unknown. OxyTx1 and OxyTx2 contain 69 and 55 amino acid residues with molecular masses of 8058.2 and 6175.2 kDa, respectively. Oxytoxins contain five disulfide bridges. OxyTx1 shows higher paralytic activity towards *Spodoptera litura* larvae.[23]

Hainantoxin-1

(HNTX-1) is an IK channel activator which shows little effect on voltage gated Na+ and Ca+ channel. M-TRTX-Hn1b (HNTX-IV) is short peptides isolated from venom of spider, *Orinthoctonus hainana* which inhibit Na+ channel Nav 1.7. This is considered as a good therapeutic targeted for pain. M-TRTX-Hn1b is equivalent to morphine that alleviates acute inflammatory pain and chronic neuropathic pain in animals and provides an attractive template for further clinical analgesic drug design (Table 1).

Zinghaotoxin

JZTX-V (Zinghaotoxin) also known as (B-theraphotoxin-CJ2a) is 29 amino acid peptide toxins which inhibit Na+ and K+ channels. JZTX-V has an inhibitory effect on TTX-S sodium current on rat DRG cells with TC50 value.[24] Jingzhaotoxin–III (JZTX-III) a 36-

residue peptide from the *Tarantula chiiobrachys* Jing2hao specific for Nav 1.5 & Nav 2.1 channels over the majority of other ion channels subtypes. The natural product argiotoxin-636 (Arg TX-636) found in the venom of the *Argiope lobata* spider is potent open channel blocker of inotropic glutamate (iGlu) receptors, and recently, two analogues, Arg TX -75 and Arg tx-48 were identified with increased potency and selectivity for iGlu receptors subtypes.[16]

Latrotoxins

L. hesperus's venom including latrotoxins, that inhibits cysteine knot toxin CRISPs, hyaluronidase, chitinase & protease & 59% of VSTs.[25] Mass spectrometry of *L.hesperus* venom identified 49 proteins from VSTs, and most of them showed 90% homology.[25] Protein content of *Parawixia bistriata* showed no phospholipase haemorrhagic or anti-leishmania activities but show low genotoxicity & discrete antifungal activity to *C. albicans*.[26] The chromatographic & electrophoretic analysis of these proteins revealed a predominance of acidic, neutral & polar proteins highlighting the presence of protein with the high molecular masses.[26] More recently, novel spider venom peptides have been found that interact with ligand gated channels (eg-purinergic receptor) and recently discovered familiar of channels & such as acid sensing ion channels, mechano-sensitive channels & transient receptor potential channels. Most peptides, the presence of an inhibitor cystine knot (ICK) in most spider venom toxins provide these peptides with extra ordinary stability[27] (Table 1).

Acid sensing ion channels (ASICs) are proton gated sodium channels that open in response to low pH. They belong to the epithelial Na^+ channels\degenerin (ENac\DEG) super family of ion channels which have the same overall topology & selectivity for transporting sodium. Functional ASIC channels comprise either homomeric or heteromeric trimers of these subunits. The only potent & specific inhibitor of ASIC 1a that has been identified to date is r-theraphotoxin Pc1a (r-TRTX-Pc 1a) which is also known as psalmotox-1 (PcTX1). A40 residues ICK peptide isolated from venom the Trinidad chevron *Tarantula jpsalmopoeus* Cambridge: r-TRTx Pc 1a inhibits homomeric ASIC 1a channels, but not other AAIC sub type, with an ICso o 0.9 nm.[8]

Therophotoxin

The venom (voltage sensor toxin) 3(VSSTX-3, K therophotoxin – Gr4a) & GTx1-1S is isolated from *Tarantula grammastola rosea*.[28] Polyamine toxins i.e. philanthoxins (phTXs) & arigotoxins have been isolated from Joro spider, these act on channel blockers of AMPARs

widely employed as highly potent antagonists of $GluA_2$ lacking receptors subtypes.[29] Family Seytodidae spider's poison contains homologues of toxic protein as tacin metalloprotease & potentially toxin proteins including venom allergen, longistalin & transnationally controlled tumor protein TCTP.[30] Spider *Vitalius dabius* contain Hyaluronidase enzyme, hyaluronic-acid containing gels indicate an absence of enzyme isoforms.[31] A tarantula venom peptide of protein -1 (proTx-1) a 35 residues peptide is isolated from the venom of the Peruvian green velvet tarantula *Thrixopelma pureins*, a well known TRPA 1 antagonist.[32] Brazilian spider *Sicarius arnatus* contains active sphingomyelinase-D (SMase-D) venom responsible for the multiple pathological effects.[33] LiD1mAb16 interact proteins of 29-36 kDa molecular weight found in *L. intermedia*, while 33 in *L-gaucho* and 27 In *L. laeta* venoms.[29] The major toxic factors in most spider venom are small, disulphide-rich peptides.[34] Dolomedes spider venom contain 100 of peptides as shown by offline RP-HPLC/MALDI- *Selenocosmia jiafu* venom contains 100 of peptides with a pre- dominant mass of 3000-4500 kDa[35] (Table 1).

Sphingomyelinase D

Loxosceles species spider main toxin is sphingomyelinase-D (SMD), a phospholipase used in generation in antibodies.[36] Two *loxosceles lata* SMD isoforms LI1 & LI2 produced in bacteria. Few spider venoms such as Lycosoidaea super family produce venom with high CP content and possess cysteine Knot are potent neurotoxins.[37] Spider venom also contains low molecular weight different classes of inorganic and organic substances such as salts, carbohydrates, amino acids, biogenic amines AP etc.[2] Spider venom contains polyamines such as spermine, spermidine, putrescine cadaverin.[38] These also possess biogenic amines like serotonin, histamine, noradrenalin, etc as well as amino acids such as glutamate, taurine, or γ-aminobutyric acids are found in venoms of many spiders Often in high concentration of the order of the tens of millimolar and higher.[39] Philantotoxins, AP in the bee walf *Philanthus triangulam* venom of wasp and spider venom components.[40] Cupiennin 1a and two latercine 1 and 2a have been isolated from spider venom.[41] Its three main fragments are distinguished in the "Canonical" structure of the knottin type spider toxins containing 6 half cystine residues. N-terminal region contains about 8-10 amino acid residues (to the second cystine residues) while central fragment is richest in half residues characterized by the highest variability both in size and amino acid composition[41] (Table 1).

The cysteine knot converts knot into hyperstable mini-protein with tremendous chemical, thermal and biological stability. ICK toxins are typically resistant to extreme of p^H, organic

solvents and high temperature[42] which selectively kills insects. There are many insecticidal neurotoxins in spider venom toxins also inhibit tumor cell proliferation and induce cytotoxicity. A novel spider neurotoxin brachyin was identified and characterized from venoms of the spider, *Brachypelma albopilosum*. Brachyin is composed of 41 amino acid residues with the sequence of CLGENVPCDKDRPNCCSRYECLEPT-GYGWWYASYYCYKKRS. There are six cysteine residues in this sequence, which form three disulfide bridges. The serine residue at the C-terminus is amidated. Brachyin showed strong lethal effects on American cockroaches (*Periplaneta americana*) and *Tenebrio molitor* (common meal beetle). This neurotoxin also showed significant analgesic effects in mice models including abdominal writhing induced by acetic acid and formalin-induced paw licking tests.

Mode of action of spider toxin peptides

The mode of action of spider toxin is potentially interact nonspecifically with cell membrane and is cytolytic in nature. Through blood stream and nerve transmission these bind to sodium and potassium gated channels and show diverse biological functions. These act as membrane disrupting agent and show cytolytic action.[43] Physiologically spider toxins are highly active which block various channels and breach the normal barrier for free movement of molecules across cell membrane. Some toxins are enzymatic in nature and hydrolyze membrane phospholipids and form channels through which small molecules may pass. Few toxins cause enormous hemolysis of RBCs and damage nerve cells. Venom toxins specifically act upon neurons, nicotinic acetylcholine receptors and neuromuscular junctions. A phospholipase toxin severely acts upon motor nerve terminals and muscle cells. It damages skeletal muscles and inhibits cell regeneration. Some protease inhibitors occur in snake venom bind to protease enzymes and prevent their action. The protease inhibitors also inhibit fibrin activity in arthritic joints and induce chronic arthritis. Various venom toxins isolated from spider venom induce immune modulatory, cardiorespiratory, analgesic and hemopoeitic activity. Venoms also cause acute and chronic inflammatory responses in laboratory animals. Histamine inhibits vaso-dilation in mast cells of lungs, liver and gastric mucosa with allergic hypersensivity and inflammation. More specifically citrate present in arthropod venom inhibits phospholipase A2 activity.[44] Several families of bioactive substances have been found from spider venoms including low-molecular weight compounds, peptides, and proteins. These venom components possess functions including induction of paralysis, activation or inhibition of ion channels and receptors, and microorganism-killing.

Toxins also change the orientation and affinity of ion binding sites and change ion permeability mediated by the nicotinic Ach receptors. Spider venom toxins block neuromuscular transmission; affect sodium and calcium exchanges and block ion channels forming a tight ring.[45] Different animal groups have different channel inhibitors. Some toxins are inhibitors of metabolic enzymes, and have hydrophobic pockets in their secondary structure by which they bind at specific substrate binding sites. The toxicity of venom depends upon the sequence of amino acid residues present in the active site regions, topological folding, hydrophobic pockets and binding affinity. Basically, site-specific mutations and rearrangements in the active site region in response to gradual environmental changes result in structural and functional diversification of toxins. Therefore, different biologically active toxins evolve within a single species. The mode of action of animal toxins and their physiological consequences vary greatly according to the structural variability in the active site region.

Few seconds after envenomation, spider toxins cause sudden inflammation in body cells with a severe pain and do massive inhibition of axonal transmission in neurons. The activity of ATP driven Na^+-K^+ ATPase pumps, which plays a key role in maintaining cell volume and intra cellular ionic composition specially Na^+ and K^+ gradients is also affected. This pump actively transports ions across the cell membrane, helps in the excitation of nerves, and does phosphorylation and dephosphorylation in muscle cells. In this mechanism some transmembrane proteins/ enzymes utilize the energy stored in molecules of ATP to move K^+ into the neuron. Na^+ -K^+ pump helps the neurons to maintain resting potential for which pump allows interior negative charge and exterior positive charge on neurons by pumping Na^+ outside the cell and K^+ inside the cell. Both Na^+ and K^+ channels are competitively blocked by these toxins, induce the release of transmitters and cause repetitive firing of the axons (Table 1).

Voltage gated ion channel interactions:

Venom-derived peptide modulators of ion channel gating are used as essential tools for understanding the molecular motions that occur during the opening and closing of ion channels.[46] Spider venoms in particular are rich in Na^+ (V) channel modulators, with one-third of all known ion channel toxins from spider venoms acting on Na^+ (V) channels. Spider venom is a potential source of new compounds with specific pharmacological properties. Voltage-gated sodium (NaV) channels play a central role in the propagation of action

potentials in excitable cells in both humans and insects. Many venomous animals have therefore evolved toxins that modulate the activity of Na(V) channels in order to subdue their prey and deter predators.[46] Voltage-activated sodium (Nav) channels are essential in generating and propagating nerve impulses, placing them amongst the most widely targeted ion channels by toxins from venomous organisms. An increasing number of spider toxins have been shown to interfere with the voltage-driven activation process of mammalian Nav channels, possibly by interacting with one or more of their voltage sensors. Spider-venom peptides that target Na (V) channels are short peptides ~6-17 kd,[13] these also partially bind to Ca^{++} ion channels and affect their ionic potential. Few of spider toxins equally affect Na^{+} and Ca^{++} currents of ventricular myocytes and work on cardiac channel antagonists.[25] Similarly, philonthotoxins (PhTXs) and argiotoxins are Ca^{2+} dependent effect of latrotoxins is caused by their tetramerization & incorporation into the presynaptic membrane with pare formation which results in Ca^{++} entry into nerve terminals are ion channels blockers of AMPARs. These are highly potent antagonists of Glu A_2-lacking receptor subtypes.[47]

Hainatoxin -1 from Chinese spider (*Ornithoctonous hainana*) more severely effect conductance of Ca activated K^{+} (IK) channels which are calcium/calmodulin regulated voltage independent K^{+} channels. These toxins put a little effect on voltage gated Na^{+} and Ca^{++} channels.[48] HNTX-IV inhibited voltage gated sodium channel NaV 1.7.[21] Similarly, PI1a Target receptors site 4 of insect's voltage gated sodium channels (nach) while Hv1a target voltage gated calcium channels and, M-918 in the channel α-subunit respectively.[12] JZTX-V shows stronger inhibitory effect on Nav 1.4 the isoform of voltage gated sodium channels. There are predominantly expressed in skeletal muscle cells as NaV1 subtype.[21] Peptide toxins with high affinity, divergent pharmacological functions, and isoform-specific selectivity are powerful tools for investigating the structure-function relationships of voltage-gated sodium channels (VGSCs)[21] (Table 1).

Similarly ω-hexatoxins target insect voltage gated calcium channel, while κ-hexatoxin target the insect calcium activated potassium channels and seem to be involved in negative selection.[37] TRTX-Cc1a is inhibitory to CaV2.3 channels. Cc1a preferentially inhibit Ba^{++} current (IBQ) mediate by L-type (CaV1.2& CaV1.3), while CaV channels are heterologous and expressed in *Xenopus oocyte*. Pro-TXI is an antagonist of voltage gated sodium (NaV) channel that contains distinct partially overlapping surface of pro-TX-I by which it binds to

these two ions channels.[35] Ssm TX-1 significantly block voltage gated K^+ channel in dorsal root ganglion neurons it has no effect on voltage gated Na^+ channels.[48] Small conductance Ca^{++} activated K^+ (SK) channels and voltage gated A-type Kv4 channels shape dendritic excitatory postsynaptic potential (EPSPs) in hippocampal Ca1 pyramidal neurons. These toxins synaptically evoked Ca^{++} influx through N-methyl –D aspartate receptors (NMDARs) activate spine SK channels, reducing EPSPs and the associated spine head Ca^{++} transient.[6] Similarly, Kv2 family "delayed-rectifier" potassium channels are widely expressed in mammalian neurons which also active relatively slow and their contribution to action potential repolarization.[21] Similarly, *S .jiafu* venom could inhibit voltage gated Na^+, K^+ & Ca^{++} channels in rat dorsal root ganglion (DRG) neurons as well as exhibited inhibitory effect on tetrodotoxin resistant (TTX-R) Na^+ current and T-type Ca^{++} current. It shows the presence of antagonists to both channel type and can be used for a valuable tool for channel inhibitors and drug development.[49]

More specifically, ion channels contribute to the regulation closer in Drosophila a model system in which Ca^{++} ions play active in cell contraction in Dorsal closer tissue as UV mediated release of gated Ca^{++} leads to cell contraction.[50] Similarly, GTx1-15 isolated from *Grammostola rosae* shown to be a potent inhibitor of tetrodotoxin (TTX) sensitive channels.[51] Spider peptide toxins,-agtoxins from *A. aparta* venom affect Ca^{+2} channels and hanatoxins from *G.vosea* venom affect K^+ channels.[3] HWTX-XI is considered to be a bi-functional toxin because it is strong trypsin inhibitor as well as a weak kv 1.1 K^+ channel blocker. "Wandering" spider of genus *Phoneutria* (ctenidae), a peptide neurotoxin affecting sodium channels (section disulphide containing peptide) (Table 1).

Naturally occurring toxins are invaluable tools for exploration of the structure and functional relationships of voltage-gated sodium channels (VGSCs).[6] Specific peptide toxins interact with voltage-gated sodium channels by regulating the activation or inactivation of targeted channels. Voltage-gated sodium channels (VGSCs; NaV1.1-NaV1.9) play critical role in controlling the function of excitable cells. Venom toxins interact to various channels cause channelopathies, including epilepsy, arrhythmia, paralytic myotonia, and pain.[21] Due to their complex physiological interaction and target specificity spider venom toxins are considered as useful tool for the investigation of the structure and function of sodium channel isoforms and for the development of various therapeutic drugs.[52] Spider toxins are extensively used to probe the gating mechanisms of voltage-gated ion channels[53] (Table 1).

Spider toxins target chemoexcitable ionotropic receptor of post synaptic membrane (Nicotine acetylcholine & glutamate). These cause inhibition of Ca^{++}dependent phospholipase i.e. protect against their own toxins.[54] Spinal voltage-gated calcium channels (VGCCs) are pivotal regulators of painful and inflammatory alterations, representing attractive therapeutic targets.[55] P2X purinergic receptors are ATP gated non-selective ion channels permeable to Na^+, K^+, & Ca^{++}.[56] Few spider toxins act on human voltage-gated ion channels, but they show promiscuity in targeting the receptors and some of them undergo revision of their "canonical" gating-modifying mode of action.[46]

Na^+ Channel inhibitors

The venom of this spider is comprised of many toxins, and the majority has been shown to affect excitable ion channels, mainly sodium (Na^+) channels. Hainantoxin-I (HNTX-I, Toxins) spider venom toxins are modulator of Na^+ channels. They retard inactivation or cause a shift in activation potential & interact with the so called receptor sites 3 & 4, the binding sites of α and b- toxins from scorpion venom respectively.[57] Huwentoxin IV from the venom of *H.schmidti* is pure blocker of Na+ channel & interacts with the so called receptor site 1 where classic inhibition tetrodotoxin and saxitoxins as well as H-conatoxins bind.[58] Two new polypeptide toxins named Hm-1 and Hm-2 were isolated from the venom of the crab spider *Heriaeus melloteei.* These toxins consist of 37 and 40 amino acid residues, respectively, contain three intramolecular disulfide bonds, and presumably adopt the inhibitor cystine knot motif. Hm-1 is C-terminally amidated and shows a low degree of homology to spider toxins agelenin and micro-agatoxin-II, whereas Hm-2 has no relevantly related peptide sequences. Hm-1 and Hm-2 were found to act on mammalian voltage-gated Na^+ channels. Both toxins caused a strong decrease of Na^+ current peak amplitude, with IC (50) values of 336.4 and 154.8 nM, respectively, on Na (V) 1.4. Hm-1 and Hm-2 did not shift the voltage-dependence of activation, nor did they change the kinetics of fast inactivation of the Na^+ currents. Both toxins negatively shift the steady-state inactivation process, and induce conformational changes in voltage-gated Na^+ channels after affinity binding[57] (Table 1). The voltage-gated sodium channel Na (v)1.7 plays a crucial role in pain, and drugs that inhibit hNa(v)1.7 may have tremendous therapeutic potential. ProTx-II and huwentoxin-IV (HWTX-IV), cystine knot peptides from tarantula venoms, preferentially block hNa(v)1.7.[15] jingzhaotoxin-II (JZTX-II) isolated from the tarantula *Chilobrachys jingzhao* venom consists of 32 amino acid residues including two acidic and two basic residues. JZTX-II had no effect on TTX-R VGSCs on rat dorsal root ganglion neurons but exerted a concentration-dependent

reduction in tetrodotoxin-sensitive (TTX-S).[6] PnTx2-6, a peptide extracted from the venom of *P. nigriventer*, causes erection in anesthetized rats and mice. There are so many molecular determinants of the interaction between the tarantula toxin huwentoxin-IV and two VGSC isoforms. Nine huwentoxin-IV residues (F6A, P11A, D14A, amino acids L22A, S25A, W30A, K32A, Y33A, and I35A) block Nav1.7 and Nav1.2 channel play important role in molecular folding and employ in specific interactions with sodium channel residues.[59] Hainantoxin-IV (HNTX-IV), isolated from the venom of the spider *Ornithoctonus hainana*, is a specific antagonist of tetrodotoxin-sensitive (TTX-S) voltage-gated sodium channels in rat dorsal root ganglion (DRG) cells. It adopts an inhibitor cystine knot motif and contains positively charged patch consisting of Arg26, Lys27, His28, Arg29 and Lys32 amino acids distributed on its molecular surface. Both Lys27 and Arg29 amino acids found critical residues for HNTX-IV binding to TTX-S sodium channels.

Huwentoxin-IV (HWTX-IV, also named Mu-theraphotoxin-Hh2a) is a typical inhibitor cystine knot peptide isolated from the venom of Chinese tarantula *Ornithoctonus huwena* and is found to inhibit tetrodotoxin-sensitive (TTX-S) sodium channels from mammalian sensory neurons.[60] This peptide binds to neurotoxin receptor site 4 located at the extracellular S3-S4 linker of domain II in neuronal sodium channels. HWTX-IV and three mutants (T28D, R29A and Q34D) and interact selectively to TTX-S sodium channels from adult rat dorsal root ganglion (DRG) neurons.[60] Hainantoxin-III (HNTX-III), a 33-residue polypeptide is isolated from the venom of the spider *Ornithoctonus hainana*. It is a selective antagonist of neuronal tetrodotoxin-sensitive voltage-gated sodium channels. It inhibits voltage-gated sodium channel Nav1.7, which has been considered as a therapeutic target for pain. M-TRTX-Hhn1b efficiently alleviated acute inflammatory pain and chronic neuropathic pain in animals and provided an attractive template for further clinical analgesic drug design.[21] Cc1a exhibited weak activity at NaV1.5 and NaV1.7 voltage-gated sodium (NaV) channels stably expressed in mammalian HEK or CHO cells, respectively[13] (Table 1).

Selective inhibitors of voltage-gated sodium (NaV) channels act as analgesics and are found potent inhibitors of human NaV1.7 (hNaV1.7).[61] Hm-3 is an insecticidal peptide toxin consisting of 35 amino acid residues from the spider *Heriaeus melloteei* (Thomisidae).[62] Typical for spider toxins, Hm-3 was found to adopt the so-called "inhibitor cystine knot" or "knottin" fold stabilized by three disulfide bonds. Its molecule is amphiphilic with a hydrophobic ridge on the surface enriched in aromatic residues and surrounded by positive

charges. Correspondingly, Hm-3 binds to both neutral and negatively charged lipid vesicles. The inhibition was voltage-dependent, and strong depolarizing prepulses attenuated Hm-3 activity. This toxin is sodium channel gating modifier from an *araneomorph* spider and features a "membrane access" mechanism of action. Its amino acid sequence and position of the hydrophobic cluster are notably different from other known gating modifiers from spider venom, all of which are described from mygalomorph species [62]

Jingzhaotoxin (JZTX)-V are short peptides having 29-35 amino acids isolated from the venom of the Chinese spider *Chilobrachys jingzhao*. These inhibit the sodium conductance and slow the fast inactivation of Nav1.5 expressed in Chinese hamster ovary (CHO-K1) cells.[63] [21] JZTX-XI significantly shifted the activation to more depolarized voltages and decreased the deactivation of Nav1.5 currents upon extreme depolarization, but only slightly affected voltage-dependence of steady-state inactivation. JZTX-V adopts an inhibitory cysteine knot (ICK) motif and has an inhibitory effect on voltage-gated sodium and potassium channels.[21] Similarly, protoxin II (ProTx II) possesses a well-defined inhibitor cystine knot (ICK) backbone region and a flexible C-terminal tail region, similar to NaSpTx III tarantula toxins.[64] Huwentoxin-IV (HwTx-IV) 1 was found to be a potent antagonist of hNav1.7 and involve a voltage-gated sodium channel and generate and conduct of neuropathic and nociceptive pain signals.[65] huwentoxin-IV (HWTX-IV), a 35-residue peptide from tarantula Ornithoctonus huwena venom, preferentially inhibits neuronal VGSC subtypes (voltage-gated sodium channels (VGSCs).[15]

K^+ Channel Inhibitors

HW11c4 is also an inhibitor relatively specific for Kv1.1 channels. HW11c24 and HW11c39 are found to be inactive on chymotrysin, trypsin, kallikrein, thrombin and ion channels. It display toxin diversification of the HWTX-XI superfamily and spider venom toxins can work as useful molecular templates of serine protease inhibitors and ion channel blockers for the development of potentially clinical applications.[66] Jingzhaotoxin-XI (JZTX-XI) is a novel peptide neurotoxin isolated from the venom of the spider *Chilobrachys jingzhao*. Two-microelectrode voltage clamp experiments had showed that the toxin inhibited Kv2.1 potassium currents expressed in Xenopus Laevis oocytes.[67] Jingzhaotoxin-XI/κ-theraphotoxin-Cj1a (JZTX-XI), and depolarization, but only slightly affected voltage-dependence of steady-state inactivation. Hanatoxin (HaTx), isolated from a Chilean tarantula, block voltage-gated potassium channel Kv2.[68] (Table 1)..

Jingzhaotoxin-XII (JZTX-XII), a 29-residue polypeptide, purified from the venom of the Chinese tarantula *Chilobrachys jingzhao* is specific for Kv4.1 channels, with the IC50 value of 0.363 microM. It interacts with the channels by modifying the gating behavior.[69] Huwentoxin XI (P68426) from *H.schmidti* spider venom is also a typical pore inhibitor of K^+ channels.[70] Huwentoxin IV from the venom of *H.schmidti* and tx1 (P17727) from the venom of Brazilian wandering spider *Phoneutria nigriventor* are pure blocker of Na^+ channel and interact with the so called receptor site 1 where classic inhibiton tetrodotoxin and saxitoxins as well as H-conotoxins bind.[58] Huwentoxin XI (2 JOT), contain k unitz motif and also found in *H. schmidti* spider venom. This motif is widely spread in nature and is specific of a number of protease inhibitor (such as the best known bovine pancreatic trypsin inhibitor or Aprotinin), K^+ channel blocker (from sea anemones and snakes) and of the peptides.[71] Hanatoxin 1 & 2 (HaTx – P56852, P56853) from *G.vosea* venom intract with the voltage sensitive domain of K^+ channel & interfere with their activation.[72] Huwentoxin XI (P68426) from *H. schmidti* spider venom is also a typical pore inhibitor of K^+ channels).[70] HXI is considered to be a bi-functional toxin because it is strong trypsin inhibitor as well as a WTX- k kv 1.1 kt channel blocker. Only a single residue in the S3-S4 linker (Glu-818 in hNav1.7) is crucial for allowing HWTX-IV to interact with the other key residues and trap the voltage sensor in the closed configuration.[15] *A. aparta*.[3] Potassium channels regulate many neuronal functions, including neuronal excitability and synaptic plasticity, contributing, by these means, to mnemonic processes. Huwentoxin XI (P68426) from *H.schmidti* spider venom is also a typical pore inhibitor of K+ channels.[70] Huwentoxin IV from the venom of *H.schmidti* and tx1 (P17727) from the venom of Brazilian wandering spider *Phoneutria nigriventor* are pure blocker of Na^+ channel and interact with the so called receptor site 1 where classic inhibiton tetridotoxin and saxitoxins as well as H-conotoxins bind.[58] Huwentoxin XI (2 JOT), contain kunitz motif and also found in *H. schmidti* spider venom. This motif is widely spread in nature and is specific of a number of protease inhibitor (such as the best known bovine pancreatic trypsin inhibitor or Aprotinin), K^+ channel blocker (from sea anemones and snakes) and of the peptides.[71]

Hanatoxin 1 & 2 (HaTx – P56852, P56853) from *G.vosea* venom interacts with the voltage sensitive domain of K^+ channel & interferes with their activation.[72] Disulphite containing peptide from spider venom is characterized by high variability of functional features. Many of the first studies spider peptide toxins, ω-agtoxins from *A.aparta* venom affecting ca^{++} channels & hanatoxins from *G.vosea* venom affecting K^+ channels have become

indispensable tools for investigation of their target.[3] Huwentoxin XI (P68426) from *H.schmidti* spider venom is also a typical pore inhibitor of K^+ channels.[70] HWTX-XI is considered to be a bi-functional toxin because it is strong trypsin inhibitor as well as a weak kv 1.1 kt channel blocker. In particular, A-type K^+ currents (IA) play a key role in hippocampal synaptic plasticity. Peptidic toxin Tx3-1, a selective blocker of IA currents, extracted from the venom of the spider *Phoneutria nigriventer*, effect on memory of mice. It is a selective blocker of IA currents that effect short- and long-term memory retention and in memory impairment[17] (Table 1).

Ca+ channel inhibitors

Ca++ ions play functional role in transmitter release from parasympathetic and sympathetic preganglionic terminals. N-type calcium channels play important roles in the control of neurotransmitter release and transmission of pain signals to the central nervous system. Their selective inhibitors are believed to be potential drugs for treating chronic pain. Huwentoxin-XVI (HWTX-XVI) is a neurotoxins specific for N-type calcium channels was purified and characterized from the venom of Chinese tarantula *Ornithoctonus huwena*. HWTX-XVI is composed of 39 amino acid residues including six cysteines that constitute three disulfide bridges. HWTX-XVI could almost completely block the twitch response of rat vas deferens to low-frequency electrical stimulation. Electrophysiological assay indicated that HWTX-XVI specifically inhibited N-type calcium channels in rat dorsal root ganglion cells (IC50 ~60 nM). The inhibitory effect of HWTX-XVI on N-type calcium channel currents was dose-dependent and similar to that of CTx-GVIA and CTx-MVIIA. However, the three peptides exhibited markedly different degrees of reversibility after block. The toxin had no effect on voltage-gated T-type calcium channels, potassium channels or sodium channels. Intraperitoneal injection of the toxin HWTX-XVI to rats elicited significant analgesic responses to formalin-induced inflammation pain.[60] (Table 1).

Release of acetylcholine (ACh) from preganglionic nerve terminals requires calcium entry through voltage-gated calcium channels. The calcium channel subtype required for ACh release varies depending on the particular ganglionic synapse. Toxins act on calcium ion channel and stop release of ACh in sympathetic pathways to this release of ACh from sacral parasympathetic preganglionic neurons requires calcium entry from both N-type and toxin-resistant calcium channels.[73] Ca^{++} dependent effect of latrotoxins is caused by their tetramerization and incarporation into the presynaptic membrane with pore formation which

results in Ca^{++} entry into nerve terminals. κ-Hexatoxin-Hv1c is a high affinity blocker (IC 50 of 2 nM) of insect BKca channel.[74] Channel block displayed a lack of voltage dependence, in contrast with many other spider toxins targeting vertebrate Kv channels.[75] Similarly, citrate was found in venom of some spiders, function does inhibition of ca^{++} dependent phospholipase i.e. protect against their own toxins.[76] Spider toxin target chemoexcitable ionotropic receptor of post synaptic membrane (Nicotine acytylcholine & glutamate), K-K^+ channels M-Nat channels (in the novel nomenclature the symbol M is used only for pore blacker white the symbol is used for modulators of these channels) Ca^{++}channels. PRTx27C3 a 4kDa neurotoxic peptides is isolated from venoms of *Phoneutria spiders.* It possesses six cysteine residues and cause moderate inhibition of L-type Ca^{++} channels.[77] δ/ω-plectoxin-Pt1a is preferentially blocks a subset of Ca^{++} channels that is apparently not required for neurotransmitter release; but it decreases threshold for Na^+ channel activation; and slows Na^+ channel inactivation.[78]

Cl^- channel blockers

Agatoxin III A (P33034) is a blocker of Cl^+ channels.[79] Chlorotoxins (C1Tx) is a small neurotoxic peptide isolated from the venom of *L. quinquestriatus it* is specific reversible inhibitor of chloride channels of rat colonial epithelial cells[80] (De-Bin and striachartz et al., 1991).[81]Agatoxin III A (P33034) is a blocker of Cl^+ channels.[79] Chlorotoxins (C1Tx) is a small neurotoxic peptide isolated from the venom of *L. quinquestriatus* is specific reversible inhibitor of chloride channels of rat colonial epithelial cells.[79] (De-Bin and striachartz et al., 1991).[81] Wasp *Ampulex compressa* venom inhibites calcium channels[82] while another from wasp *Ampulex compressa* venom inhibite synaptic transmission and blocks chloride channels in cockroach[83] (Table 1).

ProTxII, a peptide toxin recently isolated from the venom of the tarantula spider *Thrixopelma pruriens*, dose-dependently inhibited Ca(V)3.1 causing a decrease in current (81.6% +/- 3.1% at -30 mV in 5 microM toxin) and a positive shift in the voltage range of activation (+34.5 mV +/- 4.4 mV).[54] A novel peptide, named GTx1-15 preferentially inhibit T-type voltage-dependent calcium channels (Ca(v)3.1).[84] Ca^{++} dependent effect of latrotoxins is caused by their tetramerization and incorporation into the presynaptic membrane with pore formation which results in Ca^{++} entry into nerve terminals. κ-Hexatoxin-Hv1c, is most potent insecticidal toxin isolated from web spider family. It is a high affinity blocker (IC 50 of 2 nM) of insect BKca channel with a lack of effect of insect Nav, Cav, as well as other subtype

of Kv channels.[74] Spider toxins target vertebrate Kv channels.[75] N-type VGCC blockers Tx3-3 and Phα1β, respectively, isolated from the spider *Phoneutria nigriventer*, on symptomatic, inflammatory and functional changes allied to mouse cyclophosphamide (CPA)-induced haemorrhagic cystitis (HC).[55]

Phα1β from Phoneutria nigriventer shows anti- pruritic effects.[85] The sodium channel blocker ProTx I tonically blocked native and transiently expressed T-type channels in the sub- to low micro molar range with at least a ten-fold selectivity for the T-type calcium channel hCav3.1 over hCav3.3, and more than one hundred fold selectivity over hCav3.2.[86]

39-residue peptide, ω-TRTX-Cc1a (Cc1a), from the venom of the tarantula *Citharischius crawshayi* (now Pelinobius muticus) preferentially inhibited Ba^{++} currents (IBa) mediated by L-type (CaV1.2 and CaV1.3).[13] Polyamine toxins derived from spiders and wasps are use- and voltage-dependent channel blockers of Ca^{++} permeable AMPARs. The effect of the TARP γ-2 (also known as stargazin) on the inhibitory potency of three structurally different polyamine toxins at Ca^{++} permeable homomeric GluA1 AMPARs expressed in oocytes.[47] The venom of the spider Phonetic nigriventer, Tx3-4 on calcium channels coupled to exocytosis of synaptic vesicles. Tx3-4 is a potent inhibitor of calcium channels involved in the KCl-induced exocytosis of synaptic vesicles in brain cortical synaptosomes.[87] Selenocos jiafu venom exhibited inhibitory effects on tetrodotoxin-resistant (TTX-R) Na^{++} currents and T-type Ca^{++} currents[49] (Table 1).

Action on ion channel

Voltage gated Na^{+} channels (Nav) provide a current pathway for the rapid depolarization of excitable cells that is required to initiates an action potential. Spider venom toxin affects Na^{+} and Ca^{++} currents of ventricular myocytes because these are rich resources of cardiac channel antagonists.[25] Ca^{2+} dependent effect of latrotoxins is caused by their tetramerization & incarperation into the presynaptic membrane with pare formation which results in Ca^{++} entry into nerve terminals. Similarly, Polyamine toxins such as joro spider toxins philonthotoxins (PhTXs) and argiotoxins are Ca^{++} dependent ion channels blockers of AMPARs. These are highly potent antagonists of Glu A_2-lacking receptor subtypes.[47] Hainatoxin -1 isolated from Chinese spider (*Ornithoctonous hainana*) severely effect conductance of Ca^{++} activated K^{+} (IK) channels which are calcium/calmodulin regulated voltage independent K^{+} channels. These toxins put a little effect on voltage gated Na^{+} and Ca^{++} channels. HNTX-IV inhibit

voltage gated sodium channel Nav 1.7.[21] Similarly, PI1a Target receptors site 4 of insect's voltage gated sodium channels (nach) while Hv1a target voltage gated calcium channels and, M-918 in the channel α-subunit respectively.[12] JZTX-V shows stronger inhibitory effect on Nav 1.4 that other toxin types. JZTX-V isoform of voltage gated sodium channels predominantly expressed in skeletal muscle cells, with on IC50 value of 5.12 nM, compared with IC5 values of 61.7-2700 nM for other heterologous expressed NaV1 subtype.[21]

The W-hexatoxins target insect voltage calcium channel involve specific positive ion selection in an episodic fashion, whereas the κ-hexatoxin target the insect calcium activated potassium channels appear to be in negative selection.[37] ω-TRTX-Cc1a is inhibitory to CaV2.3 channels and preferentially inhibits Ba^{++} current (IBQ) mediate by L-type (CaV1.2& CaV1.3). Pro-TXI is an antagonist of voltage gated sodium (NaV) channel that contains distinct partially overlapping surface of pro-TX-I by which it binds to these two ions channels.[35] Ssm TX-1 significantly blocked voltage gated K^+ channel in dorsal root ganglion neurons with an IC50 value of 2000 nM, but it had no effect on voltage gated Na^+ channels.[48] Small conductance Ca^{++} activated K^+ (SK) channels and voltage gated A-type Kv4 channels shape dendritic excitatory postsynaptic potential (EPSPs) in hippocampal Ca1 pyramidal neurons.Synaptically evoked Ca^{++} influx through N-methyl –D aspartate receptors (NMDARs) activates spine SK channels, reducing EPSPs and the associated spine head Ca^{++} transient[6] (Table 1).

Similarly, Kv2 family "delayed-rectifier" potassium channels are widely expressed in mammalian neurons which also active relatively slow and their contribution to action potential repolarization.[21] Similarly, *S.jiafu* venom inhibit voltage gated Na^+, K^+& Ca^{++} channels in rat dorsal root ganglion (DRG) neurons as well as exhibited inhibitory effect on tetrodotoxin resistant (TTX-R) Na^+ current and T-type Ca^{++} current. It shows the presence of antagonists to both channel type.[49] Similarly, GTx1-15 isolated from *Grammostola rosae* shown to be a potent inhibitor of tedrotoxin (TTX) sensitive channels (IC50 0.007 μM for hNa (v) 1.7 & 0.12 μM for hNa (V) 1.3 channels with very little effect on TTX-resistant Na(V) 1.5 Na(V) 1.8 channels. Spider peptide toxin, ω-agtoxins from *A. aparta* venom affecting ca^{+2} channels and hanatoxin from *G.vosea* venom affecting K^+ channels.[3] HWTX-XI is a bi-functional toxin because it is strong trypsin inhibitor as well as a weak kv 1.1 K^+ channel blocker. "Wandering" spider of genus *Phoneutria* (ctenidae), a peptide neurotoxin affecting sodium channels (section disulphide containing peptide).

Biological effects

Spider venom is known to contain several distinct groups spider venom toxins showed diverse, structural and biological activity. Spider toxins show toxic implications in man and impose inflammation, necrosis and allergy. These induce delayed type hyper sensitivity, genetic susceptibility and toxic implications in man. Venom toxins from spider result in paralysis of muscles or stroke, muscles weakness associated with arterial hypertension, cardiac arrhythmias, myocarditis or pulmonary edema.[88] Venom from *Parawixia bistriata* spider shows anticoagulation, edema, myotoxicity and proteolysis ancasein, ozocollagen and fibrogen.[28] Venom of brown spider is the genus *loxosceles* contain phospholipase- D enzyme toxins that can cause serve demonecrosis and even death in humans.[89] Brown recluse spider bites result in necrotic lesion for which there is no known antidote only a proteolytic enzyme like trypsin might be effective in reducing toxicity.[90] Brown spider venom is responsible for skin necrosis with gravitational lesion spreading and occasional systemic manifestation such as intro vascular hemolysis, thrombocytopenia and acute renal failure.[91]

Spider venom toxins show multiple biological effects, neurotoxic effects with antiarrhythmic, antimicrobial, analgesic, antiparacytic, cytolytic, haemolytic and enzyme inhibitory activity. Spider venom toxins generate necrotizing skin lesions, cell necrosis, and show systemic reactions and impose death in animals.[36] Venom toxins show analgesic effect, do inhibition of voltage gated ion channels[21] and generate various channelopathies including epilepsy, arrhythmia, and paralytic myotonic effects with severe pain.[21] Larger toxins such as Latrotoxins from the infamous black widow spider (*Latrodectus mactans*) and related species induce neurotransmitter release and they have played an important role in dissecting the process of synaptic vesicles exocytosis.[92] Spider venom contains Jingzhaotoxin (JZTX)-V which is also known as β-theraphotoxin (Cj2a) which act upon voltage gated Na^+& K^+ channel and inhibit them.[21] This activity is due to presence of three hydrophobic (W5, M6, W7) and two cationic (R20 & K22) residues.[21] Similarly, polyamine toxins from spider were found potent open channel blockers of ionotropic glutamate (iGlu) receptors.[16] Similarly, venom from *Selenocosmia jiafu* contains various toxins (neurotoxins) which also inhibit voltage gated Na^+ K^+ and Ca^+ channels in rat dorsal root ganglion (DRG) neurons.[49] Contrary to this, few spider venom toxins exhibit inhibitory effects on tetrodotoxin-resistance (TTX-R) Na^+current, and T-type $Ca2^+$ current. Similarly, ω-TRTX-Cc1a, a novel tarantula venom peptide selectively, targets L-type Cav that is voltage gated calcium channel[13] (Table 1).

Ssm TX-1 significantly blocked voltage gated K+ channel in dorsal root ganglion neurons but display no effect on voltage gated Na^+ channels.[48] Though these toxins effect small conductance Ca^{2+} activated K^+ SK channel and alter the shape of voltage gated a type Kv4 channel in pyramidal neurons. More often, synaptically evoked Ca^{2+} influx through N-methyl-D aspartate receptors (NMDARs) activates spine SK channels, reducing EPSPs and the associated spine head Ca^{2+}.[6] A Kv2 Family "delayed-rectifier" K^+ channel is widely expressed in mammalian neurons that activates slowly and shows toxin mediated action potential and re-polarization under physiological condition.[21] ASIC-1A is the most abundant ASIC subunit in the central nervous system and it has the highest affinity for proton, which implicated as a novel therapeutic targets for a broad range of pathophisiological condition including pain, ischemic stroke, depression and autoimmune and neurodegradation disease such as sclerosis, Huntington and parkinsons disease.[93]

Spider toxins (JZ TX-V) inhibit ion channels present on nerve cell membrane[21] and show platelet aggregation in haemostatic system.[94] These show anti-coagulation, oedema, myotoxicity and proteolytic effects on casein, azocollagen and fibrinogen.[26] *Phoneutria nigriventer* spider venom disrupt blood brain barrier and cause neuro-inflammation in central neurons along with excitotoxic signals in rat and human. Indian ornamental tree spider (*Poecilotheria regalis*) causes severe long lasting muscle cramps after a severe bite.[95] Similarly intrathecal administration of ω-conotoxin MVIIA does lower down mechanical hyper-algesia and reduces pain induced by paclitaxel.[96] Spider *Ornithoetonus hainana* effect on neonatal rat ventricular myocitus cellular and ionic electro physiology. Its venom potentially targets action of neurotransmitters, hormones and can be used a drug for treatment of cardiac diseases.[25] Spider toxin peptides are proved much valuable tool for the investigation of structure and function of channels and for drug development.[49] (Table 1).

Neurotoxic effects

Spiders toxins show neurotoxic effects because of its components-acylpolyamines, carbohydrates,aminoacid,biogenic amines.[2]Toxins peptides such as acylpolyamine and polypeptides found in millimolar concentration binds activity to synthetic function and are biologically much active.[3] Most of the venom peptides possess disulphide bridges. Biogenic amines like serotonin, histamine etc as well as amino acids such as glutamate, taurine, or α-aminobutyric acid are found in venom of many spider.[39] Spider venom interact with Na^+, K^+, Ca^+channels and proton pumps (H^+) mechano and thermorecepter. In most of the cases

venom components work synergistically and provide efficiency affection of the mixture.[4] Intracerebroventricular administration of δ-CNTX-Pn2a into mice results in scratching, hyper-salivation,l acrimation,sweating and agitation followed by spastic paralysis of the anterior and posterior extremities and death.[97]

Neurotoxin Tx3–2 (076201) act against L-type channels, Tx3-4 Pn3-4a, w-PTx-II A; P81790), it is structurally similar to ω-agatoxins III. It irreversible inhibits P/Q & N type channels and also show action against R-type channels which is incomplete & reversible. Tx3-3 (w Pn Tx3-3;P81789) & Tx-3-6 (p81792) exhibit a broad spectrum of specificity.[98] Ca^{2+} dependent effect of latrotoxins is caused by their tetramerization and incarporation into the presynaptic membrane with pare formation which results in Ca^{++} entry into nerve terminals.

P_2X_3 binds to nociceptive sensory neurons; it causes acute and, chronic neuropathic pain, visceral pain, migraine and cancer pain.[99] Similarly, SCN9A results complete inability to smell (Hyposmia) is the only other sensory impairment in individual with this chanellopathy.[100] Spider toxins are used for treating erectile dysfunction, several drugs on the market today for treatment of erectile dysfunction (ED).[101] Spider toxin shows intermediate-conductance of Ca^{2+}activated K^+ (IK) channels mainly calcium/calmodulin-regulated and voltage-independent K^+channels (SK1/SK2/SK3 and IK.[102] There impose membrane hyperpolarization,and effect intracellular Ca^{2+} signaling. Expression of hIK1 in HEK293T cells gives rise to inwardly rectifying K^+ currents, which are activated by sub-micromolar concentrations of intracellular Ca2+ (EC50 = 0.3 μM).[103] Spider toxins generate cardiovascular effect in animal models.[104] These are also IK channel modulators mainly comprise small organic compounds and are most selective and potent ion-channel inhibitors [105] with IC-50 in the micromolar or even nanomolar range. Some small organic compounds also exhibit high affinity for IK channels, but at higher concentrations, they usually exert non-specific actions[106] but NS309 at micromolar concentrations inhibits cardiac hERG K^+ channels.[107] There toxins shows weak selectivity to some of the activators, work on at higher dosages in vitro and in vivo).[108] (Table 1).

Mu-theraphotoxin-Hhn2b, UniProtKB: D2Y1X7) is a polypeptide neurotoxin isolated from the venom of Chinese bird spider *Ornithoctonus hainana*.[109] It is composed of 33 residues and stabilized by intracellular disulfide bridges (Cys2–Cys17, Cys9–Cys22 and Cys16–Cys29), the toxin adopts a typical inhibitor cystine knot (ICK) structural motif that frequently

emerges in spider toxins and conotoxins. HNTX-I shows no effect on the neuronal TTX-S VGSCs in adult rat dorsal root ganglion neurons nor does it target VGSCs in cardiac or skeletal muscles of mammals.[109] It selectively blocks rNav1.2/β1 and para/tipE channels expressed in *Xenopus laevis* oocytes.

Cytotoxic effects

Spider venom toxins are also membrane-active peptides (MPs) which selectively act on the membranes of various cells, are among the main effectors in the "innate immunity" system, which is the earliest defense system of eukaryotes.[110] Some spider toxins peptides target membrane and show catalysis and also bind receptors to form ligand-receptor complex.[45] VStx1 toxin was isolated from *Grammostola spatulata* spider venom uses the "membrane catalysis" mechanism to interact with the voltage-sensitive domains of K^+channels localized in the cell membrane and it lacks pore-forming ability.[111]

Analgesic effects

Spider venom also does modulation of Nav channels and show killing of insect prey. Insect Nav channels share 55-60% identity with each of the vertebrate Nav subtype.[112] More specifically, preferential expression of Nav 1.7 in peripheral sensory & sympathetic neurons makes it an ideal target of novel analgesics. Other known analgesics are non-spasctic Nav channels blockers such and are used for local anesthetic agents. However, the nonspecific block of Nav channels by drug efficaciously increase toxic levels, with numerous CNS-related side effects such as dizziness and ataxia.[116] P2X purinergic receptors are ATP gated non-selective ion channels permeable to Na^+, K^+, & Ca^{++}, currently seven subunits (P2X 1.7) are known and functional P2X channels are from all by association of these subunit to form homomeric or heteromeric trimmers.[56] Spider toxins are potent inhibitors of mechanosensitive channels (MSCs).[113] Li Tx 1 interact with Na^+ or Ca^{++} Channel, but its specificity to ion channel was not determines.[87] The transcriptome analyses of *L. intermedia* venomous gland additionally revealed the presence of another class of ion channel-binding peptides[114] (Table 1).

Proteolytic effects

Crude venom and fraction P_1, P_2 and P_3 showed larvicidal effects on a *A. agepti*, fraction P_4 showed the presence of possible metalloprotease (60 kDa) that high proteolytic activity azocollagen and inhibit EDTA.[26]

Antinocepative effect

Pha 1β, a peptide from the venom of the *phoneutria nigriventer* shows antinoceptive effect.[115] N-methylated & N-hydroxylated spider poly amine toxin binds to NMDA & AMPA iglu receptor sub type sensitive antagonist Arg TX-93 & Arg TX-48[16] (Table 1).

Anti-microbial activity

Spider venom toxins also show wider antimicrobial potential against communicable disease pathogens.[116] These were found highly effective against antibiotic resistance clinically important bacterial pathogens such as *Staphylococcus aureus, Streptococcus pneumoniae* and *Enterococcus faecalis.* These were found highly effective than the conventional antibiotic drugs *in vitro* systems.[116] Such as daptomycin[117] more often, from venom of four different spiders, 40 different antimicrobial peptides (MAMPs) were isolated which showed wider antimicrobial activity to a range of microbe such as Gram positive and Gram negative bacteria as well as fungal pathogens such as *Candida albicans*.[118]

Insecticidal effects

Due to fast action on Na+ and K+ ion channels spider venoms are used as novel insecticidal agents for biopesticide engineering. Spider venoms contain a plethora of insecticidal peptides that act on neuronal ion channels and receptors. Because of their high specificity, potency and stability, these peptides have attracted much attention as potential environmentally friendly insecticides. Although many insecticidal spider venom peptides have been isolated, the molecular target, mode of action and structure of only a small minority have been explored. Sf1a, is a 46-residue peptide isolated from the venom of the tube-web spiders. egesteria florentina, is insecticidal to a wide range of insects, but nontoxic to vertebrates. In contrast to the majority of spider-derived sodium channel toxins that function as gating modifiers via interaction with one or more of the voltage-sensor domains, Sf1a appears to act as a pore blocker.[119]

African tarantula *Eucratoscelus constrictus* contain three short-loops and inhibitory cystine knot insecticidal toxins (κ-TRTX-Ec2a, κ-TRTX-Ec2b, and κ-TRTX-Ec2c) κ-TRTX-Ec2a causes the inhibition of insect delayed-rectifier K^+ currents, but only at significantly higher concentrations. κ-TRTX-Ec2a and κ-TRTX-Ec2b shows insect-selective effects, whereas the homologous κ-TRTX-Ec2c also display neurotoxic signs in mice when injected intracerebroventricularly.[120] Aps III isolated from trapdoor spider *Apomastus schlingeri* is highly neurotoxic to lepidopteran crop pests.OxyTx1 and OxyTx2 are a new family of

insecticidal peptides from spider venom.[23] Spider venom toxins block neuromuscular transmission in insects which is mediated by Glutamic acid receptor. Such toxins usually found in *Agilonopsis aperta* which are paralytic toxins. Similarly venom of Orb Webber spider (*Argiope & Nephilia*) contains synaptic toxins. There are some transitory paralytic agents α agatoxins and insectotoxin which cause repititive firing of numerous and shows massive transmitter release from pre synaptic stores at neuromuscular junction. Plecto toxins are paralytic in nature while insecticidal toxins are acylpolyamine which easily kill insects and assist hunting spider *Plecturise tristis*. Some of them act on glutametergic neuromuscular junction at post synaptic sites.

Spiders possess highly selective toxins for insects to paralyze them. Most of spider toxin peptides contain disulphide bond and exhibit neurotoxic function. They interact with Na^{+}, K^{+}, and Ca^{++} ion channels and H^{+} machano and thermo receptors. PI1a Target receptors site 4 of insect's voltage gated sodium channels (nach) in insects while Hv1a target voltage gated calcium channels and, M-918 in the channel α-subunit respectively.[12] Similarily ω-hexatoxins as target insect voltage calcium channel involved,while κ-hexatoxin target the insect calcium activated potassium channels and seem to be involved in negative selection.[37] The spider venom peptide δ-amaurobitoxin-PI1a, targets insect voltage-gated sodium channels, was fused to the "carrier" snowdrop lectin (GNA) to confer oral toxicity. The toxin (PI1a) and an amaurobitoxin/GNA fusion protein (PI1a/GNA) produced using the yeast *Pichia pastoris* as expression host show high mortality in cabbage moth (*Mamestra brassicae*) larvae. PI1a/GNA fusion protein caused 100% larval mortality within 6 days when fed to 3rd instar larvae, and caused significant reductions in survival, growth and feeding in 4th - 6th instar larvae. The PI1a/GNA fusion protein also caused mortality to dipteran (*Musca domestica*; housefly) and hemipteran (*Acyrthosiphon pisum*; pea aphid) insects and screened as a promising candidate for development as a biopesticid.[12] κ-Hexatoxin-Hv1c, is most potent insecticidal toxin isolated from web spider family. It is a high affinity blocker (IC 50 of 2 nM) of insect BKca channel with a lack of effect of insect Nav, Cav, as well as other subtype of Kv channels.[74] Hm-3 is an insecticidal peptide toxin consisting of 35 amino acid residues from the spider *Heriaeus melloteei* (Thomisidae).[62] *Sicarius* and *Loxosceles* spider venom induce both local and systemic effects in *animals*. SMase D hydrolyzed sphingomyline resulting in the formation of ceramide-1-phosphate and choline and in the presence of Mg^{2+}. It also catalyzes the release of choline from lysophosphatidylcholine. The venom of *Sicarius albospinosus* can induce systemic effects

including disseminated intravascular coagulation in rabbits.[121] Spider toxins interact to neuronal receptors and neurotransmitter and exert negative effect on neuronal activity and block synaptic transmission.[122]

The spider polyamine toxins Joro spider toxin-3 (JSTX-3) and *Nephila polyamine* toxins-1 and -8 (NPTX-1 and NPTX-8) orb-weaver spider *Nephila clavata* found in (Joro spider) found to be very potent open-channel blockers of ionotropic glutamate (iGlu) receptors.[123] Synthetic spider toxin peptides also show for their target specificity, biological selectivity with potential binding to AMPA and NMDA receptors. Polyamine toxins such as joro spider toxins, philanthotoxins (PhTXs), and argiotoxins used highly potent antagonists of GluA2-lacking receptor subtypes. A Philanthotoxin analog, PhTX-74, can distinguish among GluA2-containing AMPAR subtypes in the presence of the prototypical transmembrane AMPAR regulatory protein γ-2 or stargazing.[47] Argiotoxin-636 (ArgTX-636) found in the venom of the *Argiope lobata* spider is a potent open-channel blocker of ionotropic glutamate (iGlu) receptors.[59]

Therapeutic Effect

Voltage-gated sodium channels (VGSCs) are essential to the normal function of the vertebrate nervous system. Aberrant function of VGSCs underlies a variety of disorders, including epilepsy, arrhythmia, and pain. A large number of animal toxins target these ion channels and may have significant therapeutic potential.[59] Spider venom toxins interact with the voltage sensitive domain of K^+ channels and interfere with their interaction.[72][124] Spider venom are a rich source of ion channel blockers with therapeutic potential[125] HWTX-I relieve pain in the inflammatory joints and eliminate arthrocele to some degree.[126] It decreases concentration of tumor necrosis factor α (TNF-α) and increase the concentration of interleukin 4(IL-4) and interleukin 10(IL-10) in rat's serum. HWTX-I can also decrease the mRNA expression level of related factors of TNF-α, interleukin 1β (IL-1β) and interleukin 6(IL-6) in inflammatory pathways in rheumatoid arthritis. HWTX-I is also found effective in antinociception in the rat model of rheumatoid arthritis, which may act through its inhibition on certain inflammatory pathways.[126]

Phospholipase-D isolated from Brown spider venom belongs to a family of toxins characterized as potent bioactive agents which shows inflammatory response, platelet aggregation, endothelial cell hyperactivation, renal disorders, and hemolysis.[127] Few spider toxin peptides potentially and selectively modulate the activity of a diverse range of

therapeutic targets.[128] These include peptides target Nav channels, ASICs, MSCs, and purenergic receptors and also showed anti-malarial and anti-microbial activity. Most of these peptides contain an ICK motif, and there extraordinary stability provides a variety of delivery option for therapeutic administration. Hainantoxin-I (HNTX-I) is used as a promising template for designing new drug for cardiovascular diseases.[129] μ-TRTX Hhn 1v efficiently alleviated acute inflammatory pain and chronic neuropathic pain in animals and provide an attractive template for further clinical analgesic drug design.[21] Recombinant *Loxosceles laeta* spider toxins are used as probes while a phospholipase has been used to generate antibodies intended for medical application.[36] M-TRTX-Gr1a is likely to be a useful tool for determining the potential of MSCs as therapeutic targets for the treatment of pathologies as a diverse as cardiac Arrhythmias, spinal cord damage muscular dystrophy and Gliomas.[113] Spider *Ornithoetonus hainana* effect on neonatal rat ventricular myocitus cellular and ionic electro physiology. Its venom potentially targets action of neurotransmitters, hormones and can be used a drug for treatment of cardiac diseases.[25] Brown recluse spider bites result in necrotic lesion for which there is no known antidote only a proteolytic enzyme like trypsin might be effective in reducing toxicity.[90]

Brown spider venom is responsible for skin necrosis with gravitational lesion spreading and occasional systemic manifastation such as intravascular hemolysis, thrombocytopenia and acute renal failure.[20] P2X purinergic receptors are ATP gated non-selective ion channels permeable to Na^+, K^+, & Ca^{++}.[56] Huwentoxin XI (2 JOT), contains k unitz motif that occurs in *H. schmidti* spider venom and is widely spread in nature. There are number of protease inhibitor (such as the best known bovine pancreatic trypsin inhibitor or Aprotinin), K^+ channel blocker (from sea anemones and snakes) and of the peptides[71]

Evolutionary significance of Channel Blockers

Animal toxins have structural and evolutionary relationships between different animal groups. Due to environmental changes, site-specific mutagenesis and rearrangements diverse toxin groups are evolved. Their toxicity depends on the amino acid residues present in the active site region and topological folding occur due to temperature sensitivity and elements of earth. Modifications in active site region led to the structural and functional diversification of toxins and established different biologically activity during the long course of time. From the evolutionary point of view the voltage-gated channels are cellular adaptive structures, which display affinity to the toxin as ligands. These ion channels behave as specific gates and help

in ionic conduction through the membrane. Their opening and closing depend on electrical potential generated by specific stimulus. The cells devoid of these channels are more primitive to those bearing these channels. For formation of physiological electrochemical gradient these channels depend on ion permeability all along both sides of nerve membrane. Permeability of ions also depends on their size, types of channel and electric discharges. These channels solely depend for physiological action on electrochemical gradient formed on both sides of the membrane.[130] All these channels are found in host cells or in prey.

The divergence in evolution has generated different interrelated clusters of toxins within the families/genera. Spider toxins are much diversified and most diverged genus is *Plectreurys*, which has different toxins with variable active sites. Plectoxins is a possible ancestral protein, which has acquired most diverce mechanism of action. It is plausible that the extended family of spider toxins began to diverge from a common ancestor that had similar structure and biochemical function at a very early stage. Subsequently, the signature sequences required for the definition of basic toxic activity in most of the spider toxins acquired variable region according to the climatic conditions. In a well-defined ancestry of the Spider toxins *Plectreurys* toxin seems to be more diversified protein. Evolution of diverse defense mechanism indicates that toxins, proteins, co-enzymes are the molecules which provide protection to the animals. Overall diversification in toxins depends upon the types of tissues and environment of the animals. In different prey/ host animals pores of channels get blocked and ionic conduction inhibited for the protection against toxin action. It is one of the most highly significant molecular defense mechanism, which has developed during long evolution. Similar mechanisms have been observed in other animal groups on toxin diversification and mechanism of their physiological action. There are very few venom toxins, which impart high toxic effect on both mammals and insects; their toxic effects are very weak in comparison to anti-insect toxins inhibitors. *Hololena curta* venom shows lethal effects on cricket and ultimately caused death. It shows irreversible presynaptic neuromcucular blockage.[131] Most abundant toxin bearing species of spiders are *Plectereurys tristis* (plectoxin), *Aegelonopsis aperta* (omega-agatoxin), *Phoneutria nigriventer (*nueurotoxin*), Aptostichus schlingeri (*aptotoxin*), Atrax robustus (*robustoxin*) and Atrax versutus (*versutoxin*)* which are highly paralytic. Plectoxins are relatively small having molecular weight 5kDa).Also anti-insect toxins (cytotoxins) are found in new world spiders, these are less evolved and absent in ancient spiders. Small spider toxins, which have both beta and alpha type structures, show bifurcation of toxins into two groups. When an amino acid with

non-charged polar group responsible for non-target of channel binding is transformed into a basic amnio acid the polypeptide becomes toxic. The other types of toxins are high-grade killers containing highly charged basic amino acids in their alpha helical region. Spider txoins HNTX-IV inhibit voltage gated sodium channel Nav 1.7 [21] while PI1a Target receptors site 4 of insect's voltage gated sodium channels (nach). Hv1a target voltage gated calcium channels and, M918 in the channel α-subunit respectively.[12]

JZTX-V has much stronger inhibitory effect on Nav 1.4 the isoform of voltage gated sodium channels predominantly expressed in skeletal muscle cells, with on IC50 value of 5.12 nM, compared with IC5 values of 61.7-2700 nM for other heterologous expressed NaV1 subtype.[21] From an evolutionary point of view, the presence of anti mammal toxic effect next to anti-insect toxicity is a progressive adaptation of the venomous secretion of putative preys of spiders.

But the mode of conversion of old world toxins (alpha type) into new world beta type toxins remains unsolved to date.Brazilian Spider *Sicariusornatus* (Araneae, Sicariidae), Brown Spider(*Loxosceles intermedia*), Spider, *Brachypelmaalbopilosum*, Spider *Selenocosmia jiafu*, Chinese Spider, *Ornithoctonus hainana*, *Vitalius dubius* (Araneae.Theraphosidae) toxins are ion channel blockers[21] but are diversified according to the survival of spiders.[131] As chemical drugs were found inefficient to neutralize the toxic effects but antibodies can subside these effects very easily.

Table 1 showing important spider toxins, their nature and physiological effect in animals.

S. N.	Species Name	Nature of Toxins	Biological Effects
1	*Ornithoctonus hainana*	Apeptide toxins	Intermediate conductance Ca^{+} and activated K^{+} channels
2	*Chilobrachys jingzhao*	29 amino acid peptides	Adopt an inhibitory cysteine knot (ICK)motif, inhibitory effect on voltage gated Na^{+} and K^{+} channels
3	*Loxosceles laeta*	Sphingomyelinase D (SMD)	Necrotizing skin lesion, systemic reaction and even death.
4	*Latrodectus hesperus*	Latrotoxins, Hyaluronidase, Chitinase, Protease	Massive neurotransmitter release from vertebrate and Invertebrate neurons.
5	*Parawixia bistriata*	High molecular mass of protein.	Have potential for Biotechnological application and anticoagulation, edema, myotoxicity.

6	Ant eating spider (Araneae), Genus (Zodarion)	Very potent venom	Paralyze the prey
7	Brown Recluse spider	A highly protein content	Necrotic skin lesion, No antidot
8	*Phoneutria nigrivbenter*	Phα1β	Antinociceptive effects
9	*Cheiracanthium punctorium*	Insecticidal CpTx	Evolution of insecticidal two domain knottin toxins
10	*Selenocosmia jiafu*	100 of peptides with 3000-4500 mass	Voltage gated ion channels in rat dorsal root ganglion neurons.
11	*Dolomedes mizhoanus*	100 of peptides, a fewer high molecular mass (7000-9000)Da.	Inhibite Na^+, K^+, Ca^+, in rat DRG neurons.
12	*Dolomedes sulfurous*	Hundred of peptides	Inhibite Na^+, K^+, Ca^+ channels.
13	Australian funnel-web spider	Small disulphide rich peptides, Pharmacologically complex venom.	Target neuronal receptor and ion channels in prey.
14	Tarantula *Citharischius crawshayi*	Ω-TRTX-Cc1a	Blocker of voltage gated Ca^+ Channels
15	Peruvian green velvet tarantula	Prototoxin-1 (ProTx-1)	An antagonist of NaV channels
16	*Vitalius dubius*	Hyaluronidase	Paralytic activity
17	Lynx spider, Genus (Oxyopes)	Long peptides toxins (~110 residues).	Act on both linear and Knottin domains
18	Barychelid spider, *Trittame loki*	42-46 full length toxins precursers	Use as therapeutics and insecticides.
19	Latrodectus tredecimguttatus(Black widow spider)	Molecular composition unanswered	Paralytic activity
20	Scytodes spider	Astacin metalloprotease	Cause allergence, translationally controlled tumer protein(T CTP).
21	*Poecilotheria* (Indian ornamental tree spider)		Muscle cramps,even harmless
22	Colombian spider Pamphobeteus affnigricolor	Molecular mass 14kDa	Hemolytic activity
23	Tarantula	Jingzhaotoxin-3,(36 resaidues peptides)	Different type of voltage gated ion channels (Nav-1.5, Kv 2.5)
24	*Sicarius ornatus*	Sphingomyelinase-D	Dermonecrosis and severe ulceration
25	Fishing spider *Dolomedes mizhoanus*	Cystein Knot toxins(CKTs) and non CKTs	Acting on fish
26	Spider venom	Spider venom toxins are short peptides ~6-17 kd,	Bind to Na+ andCa++ ion channels and affect their potential. Few of spider toxins are affects Na^+ and Ca^{++} currents of ventricular myocytes and work on cardiac channel antagonists
27	*Ornithoctonous hainana*	Hainatoxin -1 from	severely effect conductance of Ca^{++}

		Chinese spider (*Ornithoctonous hainana*) more	activated K^+ (IK) channels which are calcium/calmodulin regulated voltage independent K^+ channels.
28	HNTX-IV	*Ornithoctonous hainana*	inhibited voltage gated sodium channel Nav 1.7
29	Ssm TX-1	Spider	significantly block voltage gated K^+ channel in dorsal root ganglion neurons it has no effect on voltage gated Na^+ channels
30	*S .jiafu* venom	*Toxic* venom	inhibit voltage gated Na^+, K^+ & Ca^{++} channels in rat dorsal root ganglion (DRG) neurons as well as exhibited inhibitory effect on tetrodotoxin resistant (TTX-R) Na^+ current and T-type Ca^{++} current.
31	Joro spider toxin-3 (JSTX-3) and *Nephila olyamine* toxins-1 and -8 (NPTX-1 and NPTX-8) orb-eaver spider *Nephila clavata* found in	The spider polyamine toxins	found to be very potent open-channel blockers of ionotropic glutamate (iGlu) receptors
32	*Argiope lobata* spider and its two	Analogues Arg TX-75 & Arg TX-48	ω-agatoxins IV A& IV β are usually used diagnostic ligand of p-type Ca^{++} channels
33	Spider *Vitalius dabius*	Hyaluronidase enzyme,	Hemolysis
34	*Thrixopelma pureins*,	A tarantula venom peptide of protein -1 (proTx-1)	known high affinity peptide TRPA 1 antagonist
35	Brown spider	Complex mixture of toxins	the phospholipase D, astacin like metallo proteases and inhibitor cysteine knot (ICK) peptides

CONCLUSION

Spider toxins show both structural and functional diversity and interact to various channel types and subtypes. Spider venom causes skin necrosis; induce occasional systemic manifestation such as intravascular hemolysis, thrombocytopenia and acute renal failure. These possess unique k unitz motif which is widely spread in nature. These bind to glutamate receptors and low-voltage activated T-type Ca^{++} channels and bind to large group of K^+ channels; Ca^{2+}activated K^+ channels which play important role in controlling membrane hyper polarization in vascular cells. Understanding the interactions of spider toxins with sodium channels spider toxin structural templates can be used for development of novel pain therapeutics. Spider toxins have been used extensively to probe the gating mechanisms of voltage-gated ion channels. These could become useful pharmacological tools that can be used for various therapeutic purposes. Spider toxins show high neuronal target specificity and act as potent open-channel blockers of ionotropic glutamate (iGlu) receptors. They also target

specific Na(V) channel subtypes that are considered to be important analgesic targets. Spiders possess highly selective toxins for insects to paralyze them. Most of spider toxin peptides contain disulphide bond and exhibit neurotoxic effects. Because of their target specificity, biological selectivity with potential binding to AMPA and NMDA receptors and important ion channels these can be used as good insecticidal agents. These become a good source of antimicrobial agents if its ion channel interactions are studied on different microbial cells.

Abbreviations: NMDA=, AMPA, ionotropic glutamate (iGlu, mechanosensitive channels (MSCs), membrane-active peptides (MPs), philonthotoxins (PhTXs)

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