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Research Article

Pharmacological Research Progress and Prospects of Spider Toxins especially on Ion Channels

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ABSTRACT

Spider toxins are huge combinatorial libraries composed of natural peptides and proteins with high medicinal values and different medicinal properties, including a variety of pharmacological effects like analgesic, anti-bacterial, insect repellent, anti-tumor, etc. Various toxins acting on different ion channels play different pharmacological efficacies. Along with the in-depth studies of spider toxins in recent years, this biotoxin family is expected to be more widely used in new drug discovery as well as in the treatment of various refractory diseases. Following worldwide research progress in this area, China has also made a unique and significant development in the past decades. This report gave a general review of the above-mentioned researches and the development outlook of this toxin family.

Keywords: Biotoxin, cardiovascular diseases, ion channels, natural active components, new drug R&D, pharmacological activities, spider venom.

INTRODUCTION

The spider, Araneae, is the biggest group except for insects in terrestrial animals. The Araneae is a large order of Arachnida with about 3935 genera 44906 described species all over the world [1], while in China the estimate is no less than 3,000 species. Many spiders discharge venom from their cheliceral claws in defense or hunting [2]. Spider venom is composed of diverse components, whose bioactive effects are also various. The venom can be used for local ischemia, or used as neuroprotective agents, pesticides, and analgesic drugs. Traditional Chinese Medicine emphasizes a widely applied remedy called "fight

poison with poison". Based on this conception and historically practical experiences, the medicinal spiders were recorded as "to cure all furuncle swollen, suppurative osteomyelitis corrosive sores, polypus and sarcoma" in Chen Zangqi's "Chinese Materia Medica" published at Tang dynasty. As a natural toxin, spider toxins hold rich prospects of medical application [3].

TYPE AND COMPOSITION OF SPIDER TOXINS AND THEIR PHARMACOLOGICAL EFFECTS

Type and Composition of Spider Toxins

In accordance with the role and functions, the spider toxins could be divided into three categories such as nerve poisons, poison necrosis, and mixed poison, while the most important of which is neurotoxins [4,5]. Neurotoxins can be further differentiated into polypeptides and polyamines, and in accordance with the difference in molecular size, the peptidic toxins could be subsequently divided into high molecular weight proteins and low molecular weight peptide toxins. The high molecular weight toxin protein could be represented by such as the α-latrotoxin (α-LTX) isolated from the venom of *Latrodectus tredecimguttatus*, α-latroerustatoxin (α-LCT) and five kinds latroinsectotoxins (LIT) (α-ε) [4,5], whose molecular weight is 131 KDa, 120 KDa, 120KDa, 140 KDa, 120 KDa, 110 KDa and 110 KDa, respectively [6]. Polyamines neurotoxins could be represented by Americas funnel web spider (*Agelenpsis aperta* Gertsch) venom toxin, α-agatoxins family. There typical necrosis poisonous toxin such as brown recluse spider especially *Loxosceles* spp. can produce severe local skin necrosis [7]. The mixed spider toxins, could be exampled by tarantula spider toxins and Clubiona spider toxins, which exhibit neurotoxic poisons and necrotic features.

According to difference of chemical composition, spider toxins contain a variety of constituents mainly composed of peptides and proteins, including polypeptide neurotoxin, cytotoxins, bradykinin analogs, antimicrobial peptides, enzymes (sphingomyelinase, hyaluronidase, phospholipase, isomerase) and agglutination active peptides. It also contains low molecular weight substances such as polyamines, nucleotides, amino acids, monoamines, and inorganic salts [8]. Spider venom contains so many ingredients making it possess a huge role in the pharmacological efficacies, thus providing a broad prospect to take advantage of these biotoxins for the development of new drugs.

Currently, nearly 500 spider toxins have been structurally identified, less than 1% of the speculated all spider toxins. Scrutiny on the identified toxin components, it could be generally divided into three categories according to molecular weight difference [9]. The first class indicated small molecules less than 1000 Daltons. This category includes inorganic ions/salts (Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Cl^{-}, etc.), organic acids (e.g., citric acid, lactic acid, dihydro-phenylacetic acid), glucose, amino acids, biogenic amines (e.g., histamine, spermine, spermidine, putrescine, etc.), and neurotransmitters (glutamate, aspartic acid, epinephrine, dopamine, GABAN-methyl-3,4-dihydrocarbylphenylethylamine), etc.. Vassiklevski implied that, such small molecules play a role not only as neurotransmitters and neuromodulators in an insect, but also as an antifeedant since they cause pain. Furthermore, these small molecules in spider toxins can also increase vascular permeability [10]. Category 2 means protein components possessing a molecular weight of 10 KDa or more, mainly indicates the aforementioned polymer toxins and proteases. The third category indicated the peptidic toxins with their molecular weights between 1000 Dalton and 10 KDa, these substances is most abundant in the spider toxin components, and occupies an important position on the spider toxins' bio-actions. Neurotoxins is an important part of these substances. As of 2009,

statistics results shows that among all of the peptidic toxins have been identified, 174 contains three disulfide bonds, 128 containing four disulfide bonds, 53 containing five pairs of disulfide bonds, containing 24 owns six pairs of disulfide bonds, 5 comprising seven pairs of disulfide bonds or more. In addition, only 16 peptidic toxins possess odd number of cysteine residues, which represent a small proportion of the total category.

Among various types of small molecules in the spider venom, the most thoroughly studied part is polyamines, especially the arylpolyamines. This article lists some typical spider polyamines for reference (Fig. **1**). Table **1** lists some spider toxins reported to possess medicinal activities [11-61].

Table 1.

Comparison of some important spider toxins possessing medicinal activities.

Spider species	Toxin's name	Amino acid sequence	Pharmaco-logical activity	Molecular weight (Da)
Latrodectus tredecimguttatus	α-Latrotoxin (α-LTX) [5]	EGEDLTLEEKAEICSELELQQKYVDIASNII GDLSSLPIVGKIAGTIAAAAMTATHVASGR LDIEQTLLGCSDLPFDQIKEVLENRFNEIDR KLDSHSAALEEITKLVEKSISVVEKTRKQM NKRFDEVMKSIQDAKVSPIISKINNFARYFD TEKERIRGLKLNDYILKLEEPNGILLHFKES RTPTDDSLQAPLFSIIEEGYAVPKSIDDELAF KVLYALLYGTQTYVSVMFFLLEQYSFLAN HYYEKG	Ca^{2+} ion channel	131.5K
	α-Latroinsectotoxin (α-LIT) [5]	EMSRADQCKLLAYTAVGYETVGNVAADIA SIEGANLVAAPVAAGGHLGKGLTDAAMIA MDCSSIPFEEIKEILNKEFKEMGRKLDKNT EALEHVSKLVSKTLSTVEKIRVEMREGFKL VIETIENIATKEIVFDINKIVQYFNNERENIN SRQKEEFVAKLQEPAPGNFLLYLRNSRTSE SGTLYSLLFRIIDQELAIPNNAGDNNAIQAL YALFYGTETFISIMFYLVKQYSYLAEYHYQ KG	Effective against insects	120K
	δ-Latroin-sectotoxin (δ-LIT) [5]	DEEDGEMTLEERQAQCKAIEYSNSVFGMI ADVANDIGSIPVIGEVVGIVTAPIAIVSHITS AGLDIASTALDCDDIPFDEIKEILEERFNEID RKLDKNTAALEEVSKLVSKTFVTVEKTRN EMNENFKLVLETIESKEIKSIVFKINDFKKF FEKERQRIKGLPKDRYVAKLLEQKGILGSL KEVREPSGNSLSSALNELLDKNNNYAIPKV VDDNKAFQALYALFYGTQTYAAVMEFLLE QHSYLADYYYQKG	Effective against insects	110K
Agelenpsis aperta	ω-Aga-IA [11-13]	AKALPPGSVCDGNESDCKCYGKWHKCRC PWKWHFTGEGPCTCEKGMKHTCITKLHCP NKAEWGLDW	Acting on voltage -gated calcium channels (L-type in DRG)	7.5K
	ω-Aga-IIA [12-14]	GCIEIGGDCDGYQEKSYCQCCRNNGFCS	Acting on voltage -gated calcium channels (N-type in chick synaptosomes)	10K
	ω-Aga-IIIA [11,12,14]	SCIDIGGDCDGEKDDCQCCRRNGYCSCYS LFGYLKSGCKCVVGTSAEFQGICRRKARQ CYNSDPDKCESHNKPKRR	Acting on voltage -gated calcium channels (L-,P/Q-,R-, N-type in rat brain synaptosomes)	8.5K

	ω-Aga-IVA [12,14,15]	KKKCIAKDYGRCKWGGTPCCRGRGCICSI MGTNCECKPRLIMEGLGLA	Acting on voltage -gated calcium channels (P/Q-type and P-type currents in cerebellar Purkinje neurons)	5220.39
	ω-Aga-IVB [15]	EDNCIAEDYGKCTWGGTKCCRGRPCRCS MIGYNCECTPRLIMEGLSFA	P-type VCCC blocker	5287.13
	μ-Aga-I [16]	ECVPENCHCRDWYDECCEGFYCSCRQPPK CICRNNN-NH2	Induces repetitive firing of action potennials in ventrolateral muscles of *Musca domestica*	4264
	μ-Aga-II [16]	ECATKNKRCADWAGPWCCDGLYCSCRSY PGCMCRPSS		4137
	μ-Aga-III [16]	ADCVGDGQRCADWAGPYCCSGYYCSCRS MPYCRCRSDS-NH2		4188
	μ-Aga-IV [16]	ACVGENQQCADWAGPHCCDGYYCTCRYF PKCICRNNN-NH2		4199
	μ-Aga-V [16]	ACVGENKQCADWAGPHCCDGYYCTCRYF PKCICRNNN-NH2		4199
	μ-Aga-VI [16]	DCVGESQQCADWAGPHCCDGYYCTCRYF PKCICVNNN		4159
Hadronyche versuta	ω-ACTX-Hv2a [17-19]	LLACLFGNGRCSSNRDCCELTPVCKRGSC VSSGPGLVGGILGGIL	VSCC in neurons of honeybee (Insect voltage-gate calcium)	4478
	ω-ACTX-Hv1a [18]	SPTCIPSGQPCPYNENCCSQSCTFKENENG NTVKRCD	Acting on voltage -gated calcium channels (VSCC in abdominal ganglia neurons of cockroach)	4050
	δ-Atracotoxin-Hv1 (δ-ACTX-Hv1, Versutoxin) [20] (Fig. **3**)	CAKKRNWCGKTEDCCCPMKCVYAWYNE QGSCQSTISALWKKC	Blocks inactivation of Na^+ currents in DRG (Rat dorsal root ganglion)	4847.2
	Janus-faced atracotoxin-Hv 1c (J-ACTX) [21,22]	AICTGADRPCAACCPCCPGTSCKAESNGV SYCRKDEP	Mediated blockage of K^+/Ca^{2+} channels (Inserticidal toxin)	3772.31
Atrax robustus	δ-Atracotoxin-Arl (δ-ACTX-Arl, Robustoxin) [11,20]	CAKKRNWCGKNEDCCCPMKCIYAWYNQ QGSCQTTITGLFKKC	Blocks inactivation of Na^+ currents in DRG	4854
Thrixopelma pruriens	Protoxin-I (ProTx-I) [23]	ECRYWLGGCSAGQTCCKHLVCSRRHGWC VWDGTFS	Nav1.2,1.5,1.7,1.8	3987.55
	Protoxin-II (ProTx-II) [23]	YCQKWMWTCDSERKCCEGMVCRLWCKK KLW	Nav1.2,1.5,1.7,1.8	3826.64
Ornithoctonus huwena	HWTX-I [15,24]	ACKGVFDACTPGKNECCPNRVCSDKHKW CKWKL	N-type calcium channel blocker	3764.48
	HWTX-II [15,25]	LFECSFSCEI/QEKEGDKPCKKKKCKGKWK CKFNMCVKV	Insecticidal toxin	8484.00

	Huwentoxin-IV (HWTX-IV) [26]	ECLEIFKACNPSNDQCCKSSKLVCSRKTRWCKYQI	Inhibit the neuronal tetredotoxinsensitive (TTX-S) voltage-gated N^+ channel	4113.83
	HWTX-V [27]	H2-ECRWYLGGCSQDGDCCKHLQCHSNYEWCVW DGT-COOH	Insecticidal toxin	4111.4
	HWTX-X [13]	KCLPPGKPCYGATQKIPCCGVCSHNKCT	Inhibition of rat dorsal root ganglion high threshold N- type calcium currents	2937.62
	SHLP-I (SHL-I) [28,29]	GCLGDKCDYNNGCCSGYVCSRTWKWCVLAGPW	Erythrocyte	3540.05
	HWTX-XVI [30]	CIGGGVPCAGAAPACCSGLVCLLPTLHGITTLSTTCTLL	N-type calcium channels	4437.4
	HWTX-XI [31]	IDTCRLPSDRGRCKASFERWYFNGRTCAKFIYGGCGGNGNKFPTQEACMKRCAKA	Trypsin inhibitor , Kv1.1 channel blockers	6166.2
	(double-knot toxin, DkTx) [32]	GDCAKEGEVCSWGKKCCDLDNFYCPMEFIPHCKKYKPYVPVTTNCAKEGEVCGWGSKCCHGLDCPLAFIPYCEKYR	Capsaicin receptor (TRPV1 ion channel)	
Grammostola spatulata	Hanatoxin 1 (HaTx1) [33]	ECRYLFGGCKTTSDCCKHLGCKFRDKYCAWDFTFS	Kv2.1	4114.73
	Hanatoxin2 (HaTx2) [15,34]	ECRYLFGGCKTTADCCKHLGCKFRDKYCAWDFTFS	K^+ channel	4102
Grammostola rosea	Grammostola mechanotoxin2 (GsMTx2) [35]	YCQKWMWTCDEERKCCEGLVCRLWCKRllNM	Stretch-activated channels (MSC, SAC) in Adult rat astrocytes	3934.77
	Grammostola mechanotoxin4 (GsMTx4) [35]	GCLEFWWKCNPNDDKCCRPKLKCSKLFKLCNFSF	Stretch-activated channels (MSC, SAC)	4095.90
Chilobrachys jingzhao	JZTX-I [23,36]	ACGQFWWKCGEGKPPCCANFACKIGLYLCIWSP	Kv2.1,Kv4.1, Kv4.2	3675.4
	JZTX-II [15]	GCGTMWSPCSTEKPCCDNFSCQPAIKWCIWSP	E- type calcium channel blockers	3726.35
	JZTX-III [15,36]	DGECGGFWWKCGRGKPPCCKGYACSKTWGWCAVEAP	Kv2.1, Nav1.5	3918.46
	JZTX-IV [37]	ECTKFLGGCSEDSECCPHLGCKDVLYYCAWDGTF	Inhibits current and slows the inactivation of sodium channel	3774.88
	JZTX-V [36]	YCQKWMWTCDSKRACCEGLRCKLWCRKII	Kv2.1, Kv4.1, Kv4.2	3605.73
	JZTX-VIII [38,39]	NH_2-LFECSFSCDIKKNGKPCKGSGEKKCSGGWRCKMNFCVKV-COOH	Block Ca^+ channe1 in DRG cells	4329.37
	JZTX-XI [40,41]	ECRKMFGGCSVDSDCCAHLGCKPTLKYCAWDGTF	Kv2.1, Kv4.1, Kv4.2	3726.38
	JZTX-XII [41,42]	YCQKWMWTCDSERKCCEGYVCELWCKYNL	Kv4.1	3665.4

	JZTX-XIII [36,41]	ECRWLFGGCEKDSDCCEHLGCRRAKPSW CGWDFTV	Kv2.1, Kv4.1, Kv4.2	4122.5
Acanthoscurria gomesiana	Gomesin [43]	ZCRRLCYKQRCVTYCRGR-NH_2	Affect bacteria, fungi, yeasts and eukaryotic parasites	2270.4
Lycosa singariensis	Lycocitin 1 [44]	GKLQAFLAKMKEIAAQTL-NH2	Inhibit growth of Gram-positive and Gram-negative (bacteria and fungi at micromolar concentrations	2034.20
	Lycocitin 2 [44]	GRLQAFLAKMKEIAAQTL-NH2		2340.28
	LSTX-A1 [45]	KECIPKHHECTSNKHGCCRGNFFKYKCQC TTVVTQDGEQTERCFCGTPPHHKAAELVV GFGKKIFG	Erythrocytes Anti-tumor	7335.33
Psalmopoeus cambridgei	Psalmopeotoxi n I (PcFK1) [17]	ACGILHDNCVYVPAQNPCCRGLQCRYGKC LVQV	Erythrocytes Anti malaria	3615.60
	Psalmopeotoxi n II (PcFK2) [17]	RCLPAGKTCVRGPMRVPCCGSCSQNKCT	Erythrocytes Anti malaria	2948.30
Lycosa chilea	Psalmotoxin1 (PcTx1) [46] (Fig. **3**)	EDCIPKWKGCVNRHGDCCEGLECWKRRR SFEVCVPKTPKT	Acid-sensitive ion channel (ASIC1a)	4689.45
Diguetia canities	DTX9.2 [47,48]	AKDGDVEGPAGCKKYDVECDSGECCQKQ YLWYKWRPLDCRCLKSGFFSSKCVCRDV	The voltage-dependent sodium channels of insect nerve membrane.	6371
Selenocosmia hainana (Fig. **2**)	Hainantoxin-I (HNTX-I) [49]	ECKGFGKSCVPGKNECCSGYACNSRDKW CKVLL	Kv2.1, Kv4.2	3607.22
	Hainantoxin-II I (HNTX-III) [23,50]	GCKGFGDSCTPGKNECCPNYACSSKHKW CKVYL	Kv4.2, Kv4.3	3607
	Hainantoxin-I V (HNTX-IV) [51]	ECLGFGKGCNPSNDQCCKSSNLVCSRKHR WCKYEIX	Nav TTX-S	3987.59
	Hainantoxin-V (HNTX-V) [51]	ECLGFGKGCNPSNDQCCKSANLVCSRKHR WCKYEI	Nav TTX-S	3972.57
Phoneutria nigriventer	PhTxl [15,52,53]	AELTSCFPVGHECDGDASNCNCCGDDVYC GCGWGRWNCKCKVADQSYAYGICKDKVN C	Na^+ channel blocker	8600
	PhTx2-1 [13,15,54]	ATCAGQDKPCKETCDCCGERGECVCALSY EGKYRCICRQGNFLIAWHKLASCKK	Na^+ channel blocker	5838.8
	PhTx2-5 [13,54]	ATCAGQDQTCKVTCDCCGERGECVCGGP CICRQGNFLIAAYKLASCKCK	Na^+ blocker	5116.6
	PhTx2-6 [13,54]	ATCAGQDQPCKETCDCCGERGECVCGGP CICRQGYFWIAWYKLANCKK	Na^+ channel blocker	5291.3
	PhTx2-9 [13,54]	SFCIPFKPCKSDENCCKKFKCKTTGIVKLC RW	Na^+ channel blocker	3742.1
	PhTx3-1 [55,56]	AECAAVYERCGKGYKRCCEERPCKCNIV MDNCTCKKFISE	K^+ channel	4582.93

	PhTx3-2 [55-57]	ACAGLYKKCGKGASPCCEDRPCKCDLAM GNCICK	Ca^{2+} channel	3540.84
	PhTx3-3 [56]	GCANAYKSCNGPHTCCWGYNGYKKACIC SGXNWK	Ca^{2+} channel	6300.00
	PhTx3-4 [55,56,58]	SCINVGDFCDGKKDCCQCDRDNAFCSCSV IFGYKTNCRCE	Ca^{2+} channel	8449.60
	PhTx3-5 [55,56]	GCIGRNESCKFDRHGCCWPWSCSCWHKE GQPESDVW	Ca^{2+} channel	5063.6
	PhTx3-6 [55,56]	ACIPRGEICTDDCECCGCDNQCYCPPGSSL GIFKCSCAHANK YFCNRKKEKCKKA	Ca^{2+} channel	6044.39
	PhTx4 (6-1) [59]	CGDINAACKEDCDCCGYTTACDCYWSKS CKCREAAIVIYTAPKKKLTC	Blocks inactivation of insect Na^{+} currents	5244.6
Cupiennius salei	CSTX-1 [53,60]	SCIPKHEECTNDKHNCCRKGLFKLKCQCS TFDDESGQPTERCACGRPMGHQAIETGLNI FRGLFKGKKKNKKTK	Ca^{2+} channels	8352.6
Scodra griseipes	SGTX1 [61]	TCRYLFGGCKTTADCCKHLACRSDGKYCA WDGTF	K^{+}	3776.32
Heteropoda venatoria	Heteropodatoxin1 (HpTxl) [49]	DCGTIWHYCGTDQSECCEGWKCSRQLCK YVIDW	Kv4.2	3910.57
	HpTx2 [49]	DDCGKLFSGCDTNADCCEGYVCRLWCK LCW	Kv4.2	3412.72
	HpTx3 [49]	ECGTLFSGCSTH ADCCEGFICKLWCRYERTW	Kv4.2	3599.38
Hysterocrates gigas	SNX-482 [61]	GVDKAGCRYMFGGCSVNDDCCPRLGCHS LFSYCAWDLTFSD	Cav E class (act on R-type and L-type Ca^{2+} channels)	4495.06

Pharmacological Effects

Analgesic Effect

Many biotoxins isolated from spider venom shows analgesic effects. Since most of the traditionally effective analgesic drugs containing side effects with addiction, which makes spider toxins have certain advantages in terms of new type of analgesic. Huwentoxin-I (HWTX-I), a spider peptide toxin isolated from venom of *Selenocosmia huwena* Wang (Fig. **2**) allows transmission of pain to be suppressed and thus exhibited analgesic effect. As a natural peptidic neurotoxin [24], HWTX-I (Fig. **3**) exhibited as the N-type calcium channel blocker on presynaptic membrane, which has important implications in terms of analgesic against sports injury. Tao *et al.* [62] performed solitary inflammatory pain model experiments by using of complete Freund's adjuvant in right ankle arthritis of rats. The results showed that HWTX-I can significantly improve the pain response in rats with unilateral adjuvant arthritis. The authors supposed that by inhibiting the expression of inflammatory cytokines in the spinal cord pain in rats model, by reducing its activation of primary afferent neurons, HWTX-I can improve the excitement valve, thus reducing inflammatory pain [62]. Chen *et al.* [63,64] constructed a pain model by rapid injection of formalin into the submucosa of sigmoid colon in Sprague Dawley rats, and proved that after subarachnoid administration of HWTX-I and huwentoxin-IV (HWTX-IV, Fig. **3**), the rats with acute inflammatory visceral pain showed dose-dependent inhibitory effect. HXTX-1 and

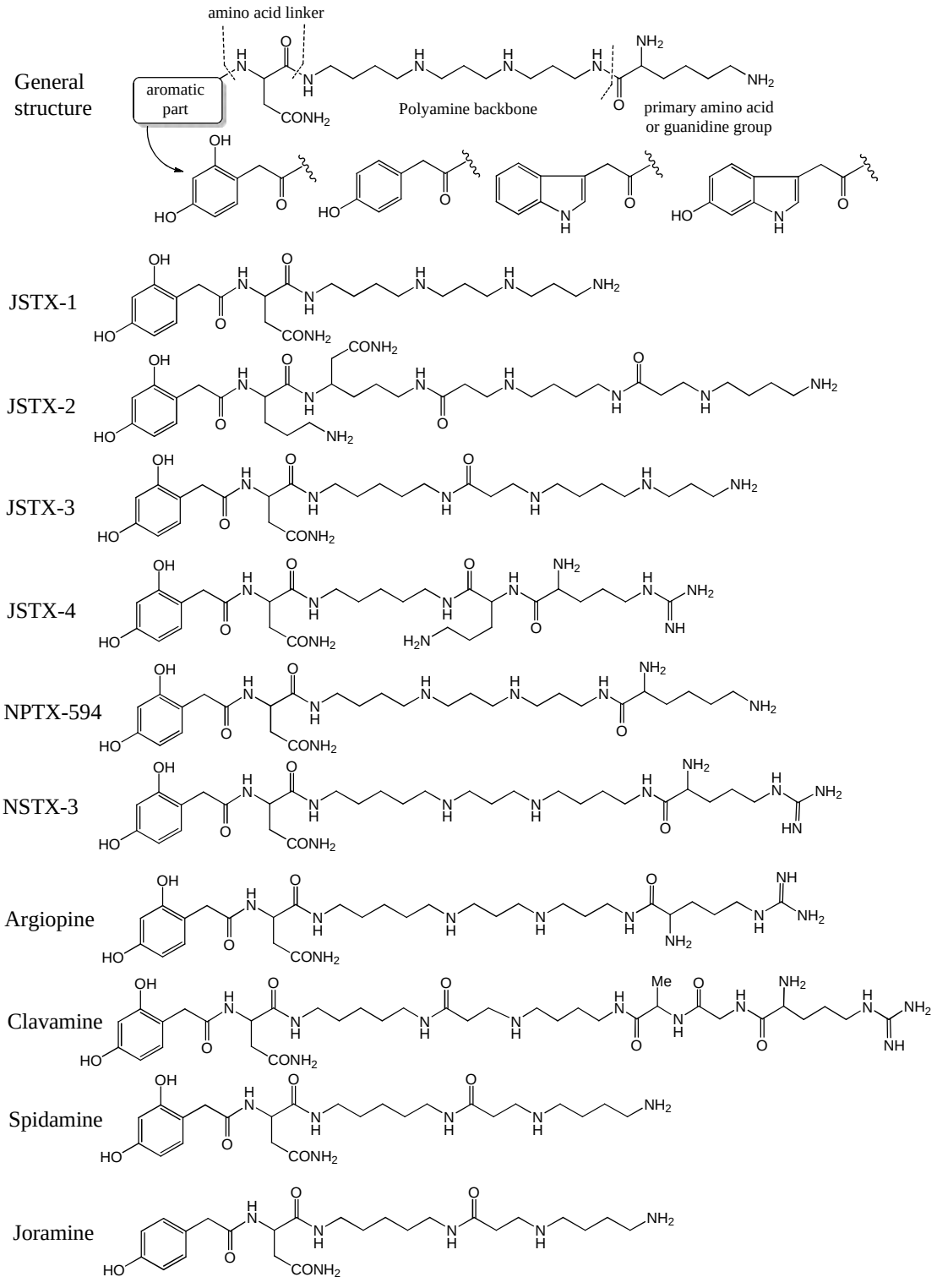


Figure 1
Chemical structures of acylpolyamines from spider venoms.

HXTX-IV show a stronger action than morphine hydrochloride, and with a longer duration. Both exhibit similar antinociceptive effects when comparing with ω-conotoxin MVIIA (SNX-111) [63,64]. In addition to Huwentoxins, Jingzhao toxins (JZTXs), a series of polypeptide toxins purified from the venom of *Chilobrachys jingzhao*, also exhibits irreplaceable analgesic activity. Zeng *et al.* [65] proved JZTX-V possesses certain analgesic effect via series of experiments such as formalin pain model, bee venom-induced inflammatory pain model, postoperative pain model, hot plate test, thermal drift experimental and scotch experiments using both epidural and intramuscular route of administration. It is reported that Zeneca Inc. declared a protein obtained from Chile tarantula also exhibit effective analgesic efficacy that can be used to relieve severe pain. This protein may play a similar role in pain inhibition with heroin formulations, but the mechanism is completely different from heroin [14].

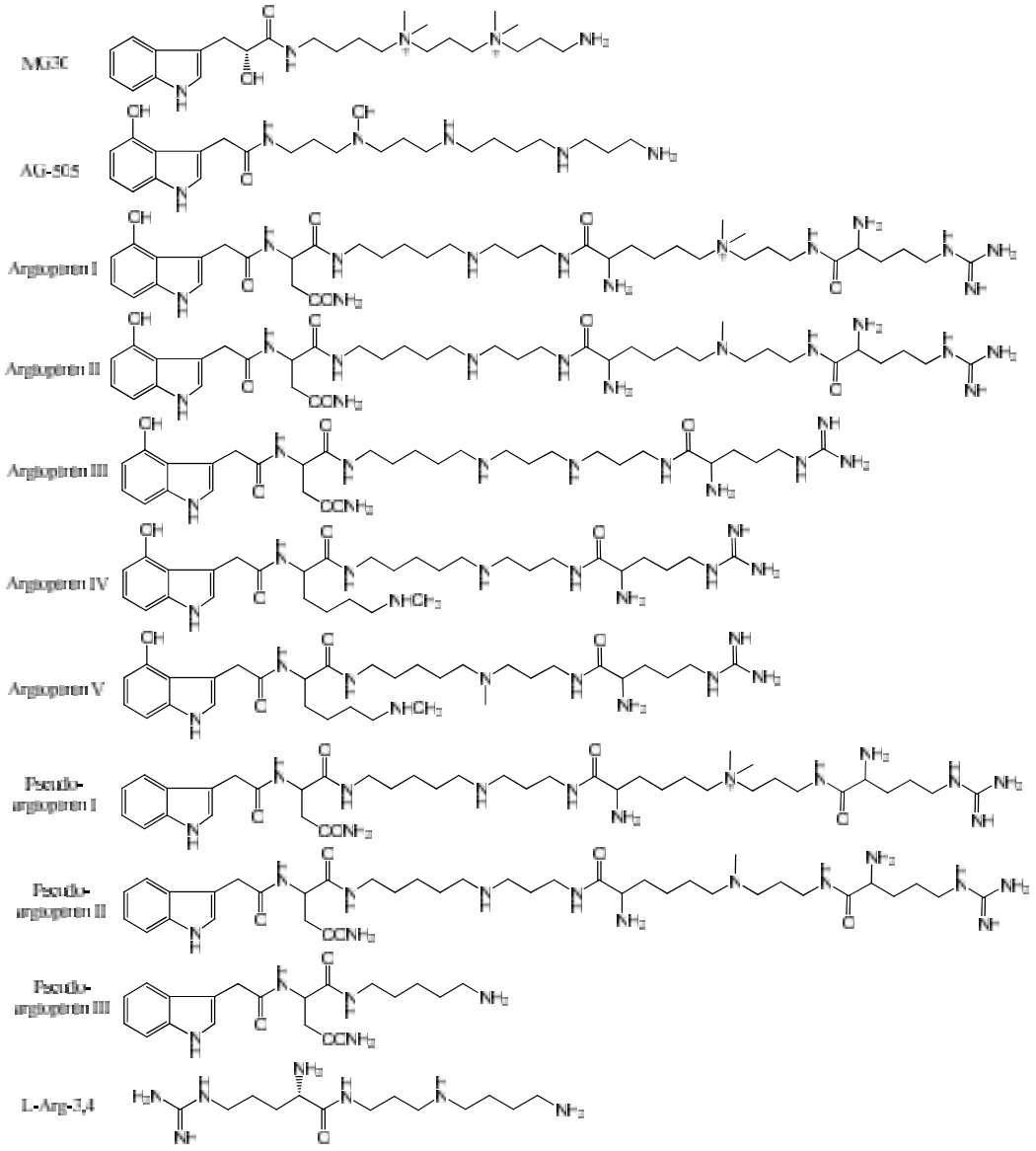


Figure 1.
Chemical structures of acylpolyamines from spider venoms. (Continued)

Latrodectus mactans Fabricius
(Black widow spider)

Nephila clavata L. Koch
(Bang Luo Xin Fu)

Selenocosmia huwena Wang
(Hu Wen Bu Niao Zhu)

Selenocosmia hainana Liang
(Hai Nan Bu Niao Zhu)

Figure 2.
Biological morphology of selected spiders with reported pharmacological efficacies.

Antibacterial Effect

Spider venom contains many antimicrobial peptides, which are poisonous natural ingredients in the spider venoms. These bioactive peptides have inhibitory effect against *Bacillus pumilus*, *Bacillus subtilis* and *Escherichia coli* [66]. From Huwena crude venom two kinds of peptidic toxins, HWTX-I and HWTX-II, was isolated, both could inhibit the growth of Gram-negative bacteria, Gram-positive bacteria and brewer's yeast, and these two peptides toxin showed a synergistic antibacterial effect [67]. Gomesin, obtained from the crude venom of Tarantula (*Acanthoscurria gomesiana*), can effectively inhibit both the growth of bacteria and the formation of hyphae of fungus, meanwhile, this peptide can also affect the survival of *Leishmania parasites* [43] The other two antibacterial peptides, Lycocition 1, lycocition 2, isolated from venom of *Burrowing tarantula* also shown to have a significant inhibitory effect of *E. coli* [44,68].

Toxicity

Spider venom secreted by the venom gland can enter into the human body through the sting wound of the skin. Spider venom contains the spider toxin hemolysin, neurotoxins and tissue toxins. Neurotoxins may stimulate the central nervous system, peripheral nerves, and autonomic nervous system causing clinical symptoms such as headache, abdominal pain, muscle pain, dizziness, salivation, weakness, convulsions, increased heart rate, coma, etc.. Tissue toxins may cause tissue necrosis, skin rash, induce chest tightness caused by myocardial necrosis, palpitation, arrhythmia, etc. [69]. If human beings are bitten by the spider, it occur local skin irritation, swelling, oozing around, getting the occurrence of necrosis, as well as the formation of ulcers. In severe cases, the bite can cause systemic toxic reactions, vomiting, high fever, convulsions and pulmonary edema embolism [70]. Furthermore, some cases will demonstrate hemolytic anemia, disseminated intravascular coagulation, and renal failure [71]. These toxic effects of spider toxins are detrimental to human, but it showed cytotoxicity against tumor cells, which can effectively inhibit the proliferation of various cancer cells. For example, a cytolytic peptide LSTX-A1, newly isolated from burrowing tarantula venom in Xinjiang province exhibit good anti-tumor effect, which can inhibit the proliferation of HeLa cells [45]. The spider venoms secreted by *Selenocosmia huwena* (Fig. **2**), *Haplopelma hainanum*, *Chilobrachy jingzhao*, *Macrothele raveni* also inhibit tumor cell activity [72-74]. The four crude spider venoms may significantly inhibit the growth of human gastric adenocarcinoma cells BGC-823 and human hepatocellular carcinoma BEL-7402 cells [75,76]. Feng *et al.* [77] found that the crude toxin of *Macrothele raveni* inhibits both the proliferation of human esophageal cancer TE-1 cell lines and the VEGF expression, while inducing apoptosis. *Chilobrachys guangxiensis*, the crude spider venom and its constituents were cytotoxic on A549 cells, human hepatomacells BEL-7402, human gastric cancer cell BGC-823, and Hela cells [74,78]. In addition, *Selenocosmia huwena* spider toxin has a significant inhibitory effect on the proliferation of glioma cell line U251 [72].

Fibrinolysis

The process of the decomposed liquefaction of fibrin formation in the blood coagulation is called fibrinolysis. The researchers isolated a new polypeptide from *Chilobrachys guangxiensis* spider venom which can play a pronounced inhibitory effect on platelet formation, and have a good anti- clotting effect, which make it a novel human platelet aggregation inhibitor [79].

Nerve-muscle junctions and Neuroprotection

Spider venom may act on the Na^+, K^+, Ca^{2+} channels, some act as activator, causing massive release of neurotransmitters, some play a role of blockers, blocking the ion channel currents thereby inhibiting neurotransmitters' delivery. Studies have shown that spider toxin can compete with Ach for the Ach receptor subunits on endplate membrane, thereby block nerve impulse transmission, and subsequent cause the loss of muscle contractility. HWTX-I is a strong irreversible inhibitor to mammalian neuromuscular transmission [24]. With their subarachnoid medication it has a neuroprotective effect on rat hippocampus in a global cerebral ischemia-reperfusion injury rat model. The mechanism was supposed to be that the HWTX-I restrain the dead signal transduction (Fas, FasL, FADD) apoptosis pathway of hippocampal neurons of rats [80]. Mao *et al.* [81] utilize global ischemic injury model of rat to observe the mophological changes by Nissel-staining of hippocampus CA1 pyramid neurons. Combined with subarachnoid catheter, they measured SOD, CAT activity and the concentration of MDA of the rat brain tissue after Nissl-staining the hippocampal pyramidal cells. The results further

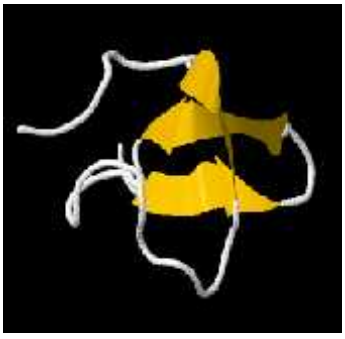
proved that HWTX-I displayed a significant protective effect on rat brain cells induced by global cerebral ischemia-reperfusion injury rat model. Mi *et al.*[82] also utilize cerebral ischemia-reperfusion injury model of mice to observe the neuroprotective effect of JZTX, and proved that JZTX has neuroprotective effect in cerebral ischemia-reperfusion injury, whose mechanism might be related to the improvement of antioxidant capacity and the down-regulation of COX-2 expressions after cerebral ischemia.

Huwentoxin-IV (4113.83 Da)

Minassian NA *et al*., *J. Biol. Chem*, **2013**; *288*: 22707-20.

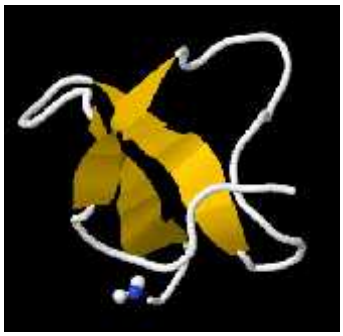
Huwentoxin-XI (6166.20 Da)

Kuan Peng K, et al., *Acta Biochim. Biophy. Sin*, **2006**, *38*: 457-66.



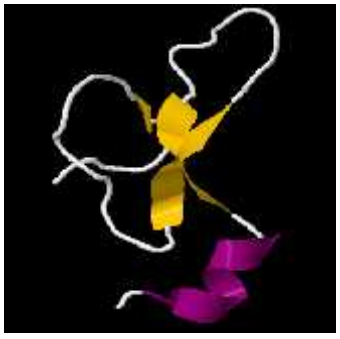
HWTX-I (3764.48 Da)

Qu Y *et al*, *J. Protein Chem*, **1997**, *16*: 565-74.



HNTX-I (3607.22 Da)

Li D *et al*, *FEBS Lett*, **2003**, *555*: 616-2.



δ-Atracotoxin-Hv1 (4862.73 Da)

Fletcher JI *et al*, *Structure*, **1997**, *5*: 1525-35.

PcTx1 (4689.45 Da)

Saez NJ *et al*, *Mol. Pharmacol*, **2011**, *80*: 796-808.

Figure 3.

Typical 3D structures of the reported spider venoms. means Beta strand; means turn; means 3/10 alpha helix.

The Role of Sensory Organs

Mammals feeling to external stimuli may not only related to stimulus intensity and manner, but also have a close relationship to some of its *in vivo* physiological conditions. The body needs to maintain a stable internal environment, in order to support bodily functions produce a corresponding sense of the appropriate stimulation and make a normal reaction. A stable pH value is the necessary factor for body to produce and transport of taste, the sense of pain, and the perception properly. The acidity of the extracellular fluid is not only directly related to the pain, but also fluctuate the perception and taste transduction with the pH changes in the brain. The changes of pH values in the brain is affected by acid-sensing ion channels (ASICs), the tarantula toxin psalmotoxin, PcTx extracted from the South American tarantula *Psalmopoeus cambridgei* and *Lycosa chilea* can block ASIC channels. Thus changing acidity of the extracellular fluid in the brain, therefore influences the generation and transmission of pain, taste and perception [83,84].

The Role of Cardiovascular and Cerebrovascular Action

The peptide GSMtX-4 purified from *Lycosa chilea* venom can effectively prevent permeability of sodium ions, potassium, and calcium ion via the specific channels located in the heart cells [85]. These ion channels are often considered to be a special biological ontogeny source of atrial fibrillation. Atrial fibrillation is a common arrhythmia disease, when the onset of the disease, the heart beats rather irregularly, which may cause blood clots and stroke. GSMtX-4 can effectively suppress and shorten the time of atrial fibrillation, therefore reduces the risk of blood clots and prevent the effects of heart disease. Another studies indicated that JZTX-I owns strong cardiac pharmacological effects.

Insect Resistants and Antigen Activity

Insects are the main prey of spiders, the spider venoms contains a variety of peptidic toxins which has been identified to be active on insects resistance [86]. All of the spider venom have toxic effects on insects, the median lethal dose LD_{50} value is rather low, which allows them killing some forest pests and agricultural pests quickly. Compared with conventional pesticides, the proteinic property of the spider venom allow them to be easily and completely denatured into the soil after decomposition, thus avoid of polluting the natural environment legacy. This leads people to realize the favorable advantage of spider toxins in agricultural utilizations, as evidenced by the Institute of Biology at the University of Queensland that spider venom peptides forms potentially extractable pesticides [87]. Moreover, introduction of the key insecticidal peptide gene into crops may enhance their resistances to pests and plant diseases, which has potentially important applications in the biological control of agroforestry.

The representative two polypeptidic pesticides are Tx4(6-1) isolated from the Brazilian spider *Phoneutria nigriventer* Keys, and DTX9.2 obtained from the Diguetidae spider *Diguetia canities*, both can cause rapid paralysis of insects, causing hyperexcitability of flies' sensory nerves and neuromuscular, as well as induce depolarization of the huge neuronal cell membrane of cockroach [59,88]. Furthermore, Gomesin, a polypeptic antimicrobial toxin isolated from the tarantula spider *Acanthoscurria gomesiana* can inhibit the *in vitro* growth of intraerythrocytic forms of *Plasmodium falciparum* [43,89]. So did two other polypeptidic toxins Psalmopeotoxin I (PcFK1) and Psalmopeotoxin II (PcFK2) exhibit, which were purified from the *Psalmopoeus cambridgei* and possesses inhibitory efficacy against *Plasmodium falciparum* [17]. Moreover, the spider toxin ω-ACTX HV2 separated from *Hadronyche versuta* is also active on the insects but invalid to mammalian. This entomological calcium channel blocker may provide a natural resource for the development of new kinds of insecticides [19]. Another study found that certain spider

venom also has the characteristic of antigen. Therefore several appropriate antitoxic serums have been prepared according to this feature, such as antitoxic serum against *Loxosceles reclusa* and antitoxic serum against *L. reclusa*.

Other Biological Effects

Selenocosmia huwena lectin-I (SHL-I), a lectin isolated from the crude venom of *Selenocosmia huwena*, which is currently the peptidic lectin possessing the smallest molecular weight [28]. Liu *et al*. identified that two peptides, m-HWTX-III and SHL-II, which was purified from the crude venom of a poisonous spider, have the same haemagglutination activity to each other [29], which can stimulate β cells and lead to insulin release. Furthermore, α-LTX, isolated from black widow spider venom also have this effect [90].

THE PHARMACOLOGICAL ACTION MECHANISM OF SPIDER VENOMS

Ion Channels

Sodium Channel

Sodium channels play an important role in the generation and regulation of sense of pain, while different types of sodium channels demonstrate different actions during the generation of various types of pain. Many pharmacological analgesic owns the same mechanism of inhibiting the neuronal sodium channel, blocking the conduction pathway of excitement, thereby reducing the sense of pain. Xiao Yu-cheng [50] observed the effects of venoms from *Selenocosmia huwena* Wang, *S. jainana* Liang, and *Macrothele raveni* on tetrodotoxin-sensitive (TTX-S) voltage-gated sodium channels (VGSCs) and delay-rectified potassium channels using undifferentiated NG108-15 cells. It was found that all of the crude spider venoms exhibited dose-dependent inhibitions against TTX-S sodium currents, but have no significant effect on outward delay-rectified potassium currents. The crude venom has the role of neuronal sodium channel blocker, but does not affect the action potential of neural stem. Its target receptors may be presynaptic membrane sodium channels. HNTX-IV (Fig. **3**) is a toxic blocker on TTX- sensitive sodium channel pore sites-1 of nerve cells, while HNTX-V displayed the similar effect on sodium channels exactly as tetrodotoxin, saxitoxin and μ-conotoxins [50,51]. Two spider polypeptidic toxins, Jingzhaotoxin-1 and Jingzhaotoxin-2, isolated from venom of the Chinese tarantula *Chilobrachys jingzhao*, both exhibit myocardial inhibition upon Nav1.5 channel current, which can be used as optional tool reagents to distinguish Nav1.5 and Nav1.8 and Nav1.9 channels [23,91]. Luo *et al*. [92] proposed Jingzhaotoxin-V can completely suppress the Tetrodotoxin-insensitive sodium channel current expressed by the rat dorsal root ganglion cells, and has a good analgesic effect. ProTx-I and ProTx-II are isolated from the venom of tarantulas *Thrixopelma pruriens*, both can reversely inhibit the tetrodotoxin-resistent sodium channel $Na_v1.8$ [93]. Derived from the venom of the Australian funnel web spider three toxins δ-ACTX-Arla, δ-ACTX-Hvl, δ-ACTX-Arlb could delay the TTX-sensitive sodium channel inactivation [94]. From Brazil *Phoneutria nigriventer* venom researchers isolated Txl, which is a reversible toxin inhibitor of the mammalian recombinant Nav1.2 channel current [95], while Tx2-6 also exhibit efficacy upon enhancement of male erectile function [96].

Calcium Channel

Jingzhaotoxin-I (JZTX-I) is an inhibitor toxin causing inactivation of calcium channel [23]. Jingzhaotoxin-III (JZTX-III) is a voltage-gated sodium channel inhibitor [15], while Jingzhaotoxin-VIII (JZTX-VIII) also showed to be a calcium ion channel inhibitor by patch clamp experiment [38,39]. In addition, indicated by patch clamp techniques, Peng *et al*. [97] found Huwentoxin-I (HWTX-I) can selectively inhibit the N-type calcium channel but showed rather weak effect on L-type calcium channel in prostaglandin E1 differentiated

NG108-15 cells. Its mechanism of action was proved to be through inhibition of presynaptic neurotransmitter's release and thereby blocking nerve conduction. The site of action was proved to be on N-type calcium channels [98]. ω-AgaIIIA is a peptide purified from the crude ω-Agatoxin of North American funnel web spider *Agelenopsis aperta*. ω-AgaIIIA can not only block the L-type and N-type calcium channel, but also act on the high voltage-activated calcium channels (P/Q-type and R-type) [99]. A cluster of spider toxin ω-ACTX-Hv1, isolated from *Hadronyche versuta*, is composed of six members, which can reversibly inhibit insect calcium channel currents, but exhibit no effect on calcium channels in mammals [100]. The half-maximal inhibitory concentration of Huwentoxin-XVI (HWTX-XVI) in rat dorsal root ganglion neurons N-type calcium channel is 60 nmol/L, which convey HWTX-XVI a highly selective blocker on the N-type calcium channels with a low toxicity and reversibility, can effectively inhibit mammalian N-type calcium channels [30]. Brazilian "armed" spider venom toxins of *Phoneutria nigriventer* Keys contains another group of calcium channel blockers, three kinds of components Txl, Tx2, and Tx3 were obtained from this venom. Tx3 type neurotoxin contains six peptidic toxins (Tx3-1~Tx3-6), intracerebroventricular injection of Tx3-1~Tx3-6 can lead to different types and degrees of paralysis, all are acting on calcium channels [54,56,58]. From Araneae spider *Cupiennius salei* another toxin CSTX-1 was isolated, which can block the L-type high threshold calcium channels on glutamate synaptosomes [101].

Potassium Channels

Huwentoxin-XI (HWTX-XI, Fig **3**) isolated from *Ornithoctonus huwenna* belongs to subfamily of Kunitz-type toxin (KTT), which act simultaneously as a serine protease inhibitor, as well as a potassium channel blocker [102,103]. Hanatoxins (including HaTx1 and HaTx2) isolated from the venom of tarantulas *Grammostola spatulata* can block the Kv2.1 channel current, whilst HaTx1 also has a weak inhibitory effect upon Kv4.2 channel [33]. The first purified toxin SGTX1 isolated from *Scodra griseipes* exhibited more than 40% and reversible blocking the fast transient potassium channel and delayed rectified mouse potassium channel in cerebellar granule cells [104]. These toxins, together with Heteropodatoxin1-3 (HpTxl-3), isolated from the spider venom of *Heteropoda venatoria* are all Kv4 channel inhibitors [49].

Regulatory Effect of Insulin Secretion

α-LTX is a proteinic neurotoxin extracted from the black widow spider (*Latrodectusm actans*) venom which can induce exocytosis of neuroendocrine cells. α-LTX stimulates β cells and causes the release of insulin. There are two key mechanisms of α-LTX promoting insulin release [105], one is perforated effect, since α-LTX embedding the cell membrane and form a permeable ion channels of Ca^{2+}, causing influx of extracellular Ca^{2+} triggers exocytosis. The other mechanism is transmembrane signal transduction. α-LTX binding to specific receptors in the membrane surface to achieve a transmembrane signal transduction, thus promote the secretory vesicles' aggregation, maturation, and membrane fusion [90].

Acting on Other Channels

Zeng *et al*. disclosed that HNTX-XXI can continually activate the transient receptor potential vanilloid-1 (TRPV1). Thereby the influx of extracellular Ca^{2+} cell was constantly kept in an excitement state, thus no longer sensitivity to external stimuli can take place, which led to analgesic anti-inflammatory effects [106]. Furthermore, the double-knot toxin (DkTx) purified from the venom of tarantula *Ornithoctonus huwena* also acts on TRPV1, kept in an ongoing liberalization state [32]. Moreover, GsMTx2 and GsMTx4, a class of peptidic toxins isolated from tarantulas (*Grammostola spatulata*) act on the mechanically sensitive ion channels [35].

THE CLINICAL APPLICATION OF SPIDER VENOM TOXINS

In recent years, the investigations of ion-channel-targeted analgesics becomes a new hot spot. Ion channels can direct regulate the neuronal excitability, participate the release of the pain media, which directly affect the generation and transmission of pain signals. Therefore, these ion channel drugs tend to have a potent analgesic effect, and have no tolerance and dependence. The common utilization of the spider toxins can be divided into two categories. One is used directly as a drug or as a precursor during molecule drug design, while another is the direct medicinal use as a research tool reagent upon various ion channels.

Partial spider venom toxins can block the opening of VGSCS, stop the sodium current, thereby chock the nervous hyperexcitability, which led to their function as analgesic drugs. The above-mentioned HWTX-I, HWTX-V, HWTX-IV, Jingzhaotoxin-III (JZTX-III), HNTX-III, and HNTX-IV all performed as this mechanism. HWTX-I, HWTX-I, in addition to its analgesic activity, it has no addictive side-effect, which make this drug does not need dose increase in the post-treatment period, and its duration of analgesia was significantly longer than that of morphine. In addition to the analgesic effect, spider toxins' formulation can also effectively withdraw from drug dependence [107]. Another medicinal promising spider venom toxin is trypsin inhibitor HWTX-XI, whose inhibitory activity is 30 times stronger than bovine pancreatic trypsin inhibitor. The scientist has successfully expressed recombinant toxin rHWTX-XI in *Saccharomyces cerevisiae* with the same activity with the wild toxin [108]. The researcher already developed it into a pre-clinical stage against pancreatitis [95,109]. In addition, the crude toxin of *Chilobrachys guangxiensis* also showed good medicinal prospects in the treatment and prevention of stroke and/or nerve damage of neurons [110]. The experimental results of the venom demonstrated significant anti-coagulation, scavenging free radicals, alleviate the symptoms of ischemic stroke, and reduce infarct size. All these support its future medical development. Moreover, two drugs developed by Chinese anti-aging research center with spider venom "Brain Regeneration Pill" and "Zeng Wei Pill-I" has achieved good results in the treatment of cerebral vascular diseases and tumors [111]. In 2006, Zhang performed the investigation of preparing the crude spider toxins of *Selenocosmia huwenna* and *Chilobrachys jingzhao* to be lyophilized powder injections as analgesic [112], while the single toxin Jingzhao toxin-V (JZTX-V) was also proved to be useful to prepare analgesic for the patients such as cancer, AIDS, intraoperative and postoperative pain, rheumatism and rheumatoid arthritis, sciatic nerve pain and trigeminal neuropathic pain [113]. In addition, Brazilian researchers described the spider venom toxin Tx2-6 from *Phoneutria nigriventer* can promote relaxation and penile erection due to NO increases, the main factor provoke an erection. It can be new drugs or combined with other existing drugs used for recovery of erectile dysfunction such as hypertension individual's erectile function [96]. Moreover, the recombinant spider protein toxin PnTx2-6-α and PnTx2-6-β can also be used to treat male erectile dysfunction. In a number of hospitals in Brazil, Argentina and Israel, doctors have conducted years of clinical application, with spider toxin treatment of male ED [114]. American Neurex Corporation identified SNX-482 isolated from an African spider venom has been demonstrated as an R-type calcium channel selective inhibitor which can be used in the treatment of neurological disorders and severe anxiety disorders.

Modern investigation indicated that the biological peptidic toxin may bind either with the receptors on the target cell membrane, or with specific proteins' domain of the ion channels, inducing activation or modification of the receptors or ion channels, and thereby generating symptoms such as shock, numbness, pain and even death [61]. Therefore, they can serve as good tools to study membrane receptors and channel structures as well as their functionalities. For

example, the double-knot toxin is a satisfactory tool used to investigate the conformation and functionalities of TRPV1 [32]. As a reagent to be used in the ion channel tools, the toxins from the American Dipluridae spider, ω-agatoxin IV A, was chosen to identify calcium channels due to its capability of selectively inhibiting P/Q-type calcium channels. Jingzhaotoxin-III possesses high affinity and selectivity on voltage-gated cardiac sodium channel subtypes, therefore make it promising to be a powerful research tool for cardiac sodium channel [9]. As a novel peptide from spider venom which can influence TTX-S sodium channel, HNTX-IV also owns great significance to rich the toxicology types of spider toxins. Meanwhile, HNTX-IV provides an important tool agent to elucidate the relationships concerning structures and functionalities of the sodium channels [50].

DISCUSSION AND OUTLOOK

Chinese traditional medicine recorded medicinal use of spider began in 739 years AD in "Herbal Supplements (Ben Cao Shi Yi)" (Tang dynasty). In Ming Dynasty (AD 1578) the "Compendium of Materia Medica (Ben Cao Gang Mu)" written by Li Shi-zhen also recorded a number of prescription medicine of spiders. The above description of the spider species of precious books are mainly distributed in five families with eight species, which are *Latouchia cornuta* Song et Qiu and *Latouchia davidi* Simon (Ctenizidae), *Uroctea compactilis* Koch and *Uroctea lesserti* Schenkel, *Araneus ventricosus* L. Koch and *Argiope bruennichii* Scopoli (Araneidae), *Agelena labyrinthica* Clerck (Agelenidae), and *Menemerus confusus* Boes. et Str. (Salticidae) [115]. According to entry in the record of the spider in the 1977 book "Chinese Dictionary", this medicinal animal has been mainly used in the civil clinical at the following applications: (1) foxy hernia, (2) stroke deviated facial paralysis, (3) chronic infantile convulsion, children with lockjaw, infantile malnutrition, (4) Furuncle swollen, scrofula, skin and external diseases, (5) sting by centipede, wasp, and scorpion [116].

Seen in this light, although the spider has not been listed as one of traditional Chinese "the five poisonous creatures" (the five poisonous creatures, scorpion, viper, centipede, house lizard, and toad), but the virulence of its pharmacological sense is significantly higher than several other poisonous animals, such as scorpions, centipedes, Wasps and so on. According to theory of traditional Chinese medicine, "fight fire with fire (counteract one toxin/poison with another)", spiders are indeed worth of further in-depth study. In the period of construction of the National-local Joint Engineering Research Center of Entomoceutics" at Dali University, this center has collected more than 36,000 head spiders from the Yunnan-Guizhou Plateau area, thus forming the largest Germplasm Bank spider in Southwest China. From the more than 1,200 identified species of spider, more than 50 new species have been already found [117-120]. There are more than 200 kinds of new species by estimation whichneed through identification before publication. The "National-local Joint Engineering Research Center of Entomoceutics" (NJERCE, Yunnan, China) continuing to establish cooperation between various disciplines of pharmaceutical spider R&D with domestic and foreign institutions, and has developed and performed a series of pharmacological screenings with these spiders extracts against cardiovascular and cerebrovascular diseases, cancer, endocrine diseases, analgesic, fungal infections, and viral diseases. Numerous exciting findings have been achieved from these spider extracts or single components, which is hopeful for the development of new drugs and are undergoing further animal experiments [121-123].

CONFLICTS OF INTEREST

The authors confirm that this article contents has no conflict of interest. This work was supported by IRTSTYN (2010-ZY-011), the P-MOST Programme for Yunnan Innovative Research Team, Key Projects

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ABBREVIATIONS

Ach = Acetylcholine
AIDS = Acquired immunodeficiency syndrome
ASICs = Acid-sensing ion channels
CAT = Catalase
COX-2 = Cyclooxygenase-2
ED = Erectile dysfunction
FADD = Fas-associated death domain, Fas-associating protein with a novel death domain
Fas = Factor associated suicide, The apoptosis-associated factors
FasL = Fas Ligand
GABA = gamma-aminobutyric acid
KDa = Killo dalton
LD50 = Median lethal dose
MDA = Malondialdehyde
NO = nitrogen monoxide
TTX-S = Tetrodotoxin sensitive
TRPV1 = Transient receptor potential 1 channel
VEGF = Vascular endothelial growth factor
VGSCs = Voltage-gated sodium channels
SOD = Superoxide dismutase

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