Peptide-rich venom from the spider *Heteropoda venatoria* potently inhibits insect voltage-gated sodium channels

Yazhou Huang^{1,#}, Xinzhou Wu^{1,#}, Peng Zhang¹, Zhigui Duan¹, Xi Zhou¹, Minzhi Chen¹, Athar Farooq¹, Songping Liang^{1,2}, Zhonghua Liu^{1,2,*}

¹*The National & Local Joint Engineering Laboratory of Animal Peptide Drug Development, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, China;* ²*The Key Laboratory of Protein Chemistry and Developmental Biology of the Ministry of Education, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, China*

* Corresponding author: Zhonghua Liu, liuzh@hunnu.edu.cn

The authors contributed this work equally

Abstract

Heteropoda venatoria is a venomous spider species distributed worldwide and has a characteristic habit of feeding on insects. Reverse-phase high-performance liquid chromatography and matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry analyses revealed that *H. venatoria* venom contains hundreds of peptides with a predominant molecular weights of 3000-5000 Da. Intra-abdominal injection of the venom had severe toxic effects on cockroaches and caused death at higher concentrations. The LD₅₀ was 28.18 µg/g of body weight in the cockroach. It was found that the venom had potent inhibitory effect on voltage-gated sodium channels (VGSCs) in *Periplaneta americana* cockroach dorsal unpaired median (DUM) neurons with an IC₅₀ values of 6.25±0.02 µg/mL. However, 100 µg/mL venom only partially blocked VGSC currents in rat dorsal root ganglion cells, a much lower inhibitory effect than that on DUM VGSCs. Our results indicate that the venom of *H. venatoria* contains diverse neurotoxins that might become new leads for bioinsecticides .

Keywords: *H venatoria* venom, dorsal unpaired median neurons, voltage-gated sodium channels, insecticides

Abbreviations: DRG, dorsal root ganglion; DUM neurons, dorsal unpaired median neurons; HEPES, N-hydroxyethyl piperazine-N-ethanesulfonic acid; LD₅₀, median lethal dose; IC₅₀, half maximal inhibitory concentration; MALDI-TOF, matrix assisted laser desorption/ionization-time of flight; RP-HPLC, reverse phase-high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; VGSCs, voltage gated sodium channels; Kv channels, voltage-activated potassium channels

1. Introduction

Spiders are the most diverse and successful terrestrial invertebrates which can capture prey and defend themselves with their venom glands (Saez et al., 2010). 46058 different species in approximately 114 different families are identified so far. *Sparassidae* comprises *Heteropodinae* Thorell, *Sparassinae* Bertkau and *Sparianthinae* Simon, which are distributed in 86 families, approximately 1208 species ((Ramírez, 2014) World Spider Catalog, version 17.0. American Museum of Natural History, available online at <http://wsc.nmbe.ch>). *H. venatoria* is a species belonging to family *Sparassidae*, which include some common indoor spiders. The spider mainly feeding on cockroaches, flies, moths and other insects is a beneficial arthropod of the public. It is useful to control pests in home or garden.

The control of agricultural and pathogenic insect pests is an important public issue in the world, which is critical for optimizing crop production as well as control of insect pests and human disease propagation (Gunnell et al., 2007; Oerke and Dehne, 2004; Pimentel, 2009). Chemical insecticides are still the dominant method for controlling insect pests in both the agricultural and the public health areas (Attaran et al., 2000; King and Hardy, 2013; Soderlund and Bloomquist, 1989). However, insecticide resistance and potential environment pollution have dramatically affected insecticide availability (Bass and Field, 2011). The development of biological control methods with high safety and efficacy has become an urgent issue (King and Hardy, 2013). In recent years, natural products have been considered as a potential source of novel insecticides (Smith et al., 2013). In particular, there is increasing interest in the potential of insecticidal peptides isolated from the venoms of insect predators including scorpions and spiders (Gurevitz et al., 2007; Windley et al., 2012).

Spider venom is a complex cocktail containing salts, organic compounds, peptides and proteins (Kuhn-Nentwig et al., 2011; Rash and Hodgson, 2002). Studies have confirmed that peptides are the most abundant component of most spider venoms and exhibit great diversity (Vassilevski et al., 2009). Spider venom peptides have different biological functions, including neurotoxic, enzymatic, cytotoxic, insecticidal, antimicrobial and hemagglutinin activities (Liang, 2008). To date, approximately 1523 peptide toxins from 97 spider species have been described in ArachnoServer 2.0 (www.arachnoserver.org), a curated database containing available information on spider-venom peptides and proteins (Herzig et al., 2010; King et al., 2008). Most spider venom peptides investigated so far are neurotoxins targeting various voltage-gated ion channels, many of which are valuable targets for drug and insecticide discovery (Bosmans and Swartz, 2010). Considering the spider *H. venatoria* mainly feeds on insects, the venom is expected to be toxic to insects and contain peptides acting on voltage-gated ion channels in insects. In a previous research, two peptides derived from the venom were found to block Kv4.2 potassium channels (Sanguinetti et al., 1997). However, the activity of the venom on insects and insect ion channels remains unexplored so far. In the present study, we collected crude venom from the spider and conducted a preliminary study of the crude venom, showing the venom is composed of hundreds of peptides and potentially inhibits VGSC

currents expressed in cockroach DUM neurons.

2. Materials and methods

2.1. Venom collection

H. venatoria (Fig. 1) were captured in the field near corners and eaves of old houses in central China, maintained in individual terrariums in our laboratory, fed weekly with mealworms and water. The venom was obtained by electrical stimulation using a method reported in our previous study (Yuan et al., 2007). The venom was frozen and stored at -80°C before use.

2.2. Peptide and protein analysis

Protein content of the venom was determined using the Bradford method (Bradford, 1976). SDS-PAGE of the venom was conducted according to the method of Laemmli. (Laemmli, 1970). The venom solution was analyzed by SDS-PAGE (15% separation gel and 5% stacking gel) using standard protocols to check the molecular weight (MW) range and stained with Coomassie brilliant blue G-250 (Sigma-Aldrich, St. Louis, MO, USA). The venom solution was centrifuged for 5 min at room temperature (25°C) and 5000 g, and then the supernatant was loaded into the gel well. SDS-PAGE was run at 15 mA on polyacrylamide stacking gel and at 25 mA on separating gel.

2.3. Reverse-phase high-performance liquid chromatography (RP-HPLC) analysis

The crude venom was analyzed on a reversed-phase C18 column (4.6 mm×250 mm, Sepax Technologies, USA). Venom components were eluted using a linear acetonitrile gradient (15–65% acetonitrile/0.1% TFA in 50 min) at a flow rate of 1.0 mL/min. Fractions were collected manually by monitoring the absorbance at 215 nm. Each fraction was collected, lyophilized and stored at -20°C.

2.4. Mass spectrometric analysis

The venom powder was dissolved in water to a final concentration of 10 mg/mL, centrifuged at 14,000 rpm for 10 min at 4°C and filtered using a 0.22 µm microfilter (MILLEX GP, Millipore). The analysis was performed using aAB Sciex-TOF/TOF 5800 mass spectrometer (Applied Biosystems, USA) by choosing the matrix α -cyano-4-hydroxycinnamic acid (CHCA) (Sigma-Aldrich, USA), 0.5 µL (10 mg/mL) mixed with 0.5 mL venom sample (10 µg/mL). Mass spectra were recorded under the control of TOF/TOF series explorer software (Applied Biosystems, USA). MALDI-TOF spectra were recorded in the positive ion linear mode over a mass range of 1000–20,000 Da. Linear final detector voltage was 1.92 kV. Laser intensity was 3800. An external mass calibration was done daily.

2.5. Bioactivity assays

Fifty-four cockroaches were randomly divided into 9 groups. Eight groups were used as experimental groups to which 10 µL of venom solution was injected between

the fourth and fifth sternites as single doses of 8.125, 11.7, 16.84, 24.25, 34.89, 50.24, 72.34 or 104.17 $\mu\text{g/g}$. The ninth group was used as control and injected with insect physiological saline. Lethality in *P. americana* was observed 24 h after injection. The LD₅₀ values were determined based on the lethality in six animals at each dose level (Liu et al., 2016).

2.6. Electrophysiological studies

2.6.1. Acute isolation and primary culture of neurons

Dorsal root ganglion neurons were acutely dissociated from 180 to 200 g adult old Sprague-Dawley rats and maintained in short-term primary culture according to the method described by Xiao and Liang (Xiao and Liang, 2003). Briefly, dorsal roots and spinal nerves were removed from the spinal cord and transferred immediately into ice-cold Dulbecco's Modified Eagle's Medium (DMEM). Dorsal root ganglions were stripped of their connective tissue capsules, transferred into another ice-cold DMEM aliquot and minced with scissors. They were transferred into Dulbecco's modified Eagle's medium (DMEM) containing trypsin (0.5 mg/ml, type III, Sigma) and collagenase (1.0 mg/ml, type IA, Sigma), and then incubated at 37 °C with gentle agitation for 30 min. Trypsin inhibitor (1.5 mg/mL, type II-S, Sigma, USA) was used to terminate enzyme treatment. After transferring into 35 mm culture dishes (Corning, Sigma), coated with 1 mg/mL poly-L-lysine (Sigma), the DRG cells were incubated for 2-4 h before patch clamp experiments.

Experiments were carried out on single dorsal unpaired median (DUM) neurons acutely isolated from the adult *P. americana* cockroaches (Wang et al., 2012). Adult cockroaches were desheathed, and their abdominal ganglia were removed and then incubated in insect physiological solution (in mM): 90 NaCl, 6 KCl, 2 CaCl₂, 2 MgCl₂, and 10 HEPES, 140 glucose, pH 6.6, containing 1 mg/mL trypsin for 5 min. Following this, the ganglia were removed and stored in physiologic solution for 1 h for restoration. For isolation of DUM cells in the dorsal midline of ganglia thin silver needles were used. The cells which appear bright under phase contrast microscope were considered as viable cells and qualified for further experimental procedures.

2.6.2. Patch clamp electrophysiology

Sodium currents were recorded using an EPC-10 patch clamp amplifier (HEKA Electronics, Germany) at room temperature (20–25°C). For VGSC currents recording, the pipette solution for rat DRG neurons contained (in mM): 145 CsCl, 4 MgCl₂·6H₂O, 10 HEPES, 10 EGTA, 10 D-Glucose, 2 ATP (pH7.2), and the external solution contained (in mM): 145 NaCl, 2.5 KCl, 1.5 CaCl₂, 1.2 MgCl₂·6H₂O, 10 HEPES, 10 D-Glucose (pH 7.4). The external solution for insect DUMneurons contained (in mM): 80 NaCl, 30 TEA.Cl, 2 CaCl₂, 4 KCl, 10 HEPES, 10 glucose, 50 choline-Cl, and 1 4-AP (pH 6.8), and the micropipette internal solution for these neurons contained (in mM): 140 CsF, 2 MgCl₂, 10 EGTA, and 10 HEPES (pH6.8). Suction pipettes (2.0-4.0 M Ω) were prepared using borosilicate glass capillary tubes with a two-step pulling on a vertical micropipette puller. To eliminate the influence of differences in osmotic pressure, all

internal and external solutions were adjusted to 280 ± 5 mosmol/L with sucrose. Experimental data were collected and analyzed using the program Pulse/Pulsefit 8.0 (HEKA Electronics, Germany) and Sigmaplot 10.0. Series resistance was maintained near 5 M Ω and compensated 65-70%. Linear capacitive and leakage currents were digitally subtracted using the P/4 protocol. All data are presented as means $\pm$ S.E., and n is the number of independent experiment.

3. Results and discussion

3.1. Venom composition

The spider *H. venatoria* can expel venom from the chelicerae after electrical stimulation. The venom is a clear and colorless liquid, easily soluble in water. Each *H. venatoria* spider yields approximately 2–15 μ L venom and the venom density was determined to be 978 mg/mL. The quantitative determination by Bradford assay suggested that the protein content of the venom was 32.34% (Table 1).

Spider venoms are commonly composed of proteins, peptides, low molecular mass organic compounds and salts with a plentitude of peptides (Kuhn-Nentwig et al., 2011). To explore the molecular weight distribution of the venom proteins, the venom was analyzed by SDS-PAGE using standard protocols. Except for polypeptides with molecular weights below 10 kDa, the venom proteins were mainly distributed in three bands on SDS-PAGE, corresponding to the molecular weights of approximately 35 kDa, 50 kDa and 70 kDa, respectively (Fig. 2A).

A typical RP-HPLC chromatogram of the venom is shown in Figure. 2B. We collected approximately 40 fractions based on absorbance at 215 nm. Most of the fractions were eluted with the retention time between 15 and 30 min, corresponding to 25–40% acetonitrile. This RP-HPLC profiling of the venom is similar to those of some typical spider venoms. Most spider venom peptides have charged or hydrophilic amino acid residues distributed on their molecular surfaces and therefore are usually eluted by low or moderate concentrations of acetonitrile. The peptide mass fingerprint of the crude venom was next determined by using MALDI-TOF-MS. As shown in Figure. 2C, just about 10 peaks were observed in the mass range from 1000 to 10,000 Da, even less than the peak number in RP-HPLC chromatogram, indicating a majority of peptides in the crude venom could not be detected. This might be explained as follows: (1) the venom contains many salts and low molecular mass compounds, which may interfere with the ionization of peptides; (2) many peptides are present in very low amounts, which are not able to be detected (Escoubas et al., 2006). Only three mass peaks (3276.5496 Da, 3207.5718 Da and 3920.4082 Da) were readily detected in each examination, suggesting these peptides might be the main components of the venom and suppress the signals of many low amount components in MALDI-TOF-MS analyses.

Subsequently, the HPLC fractions were individually submitted to MALDI-TOF MS analysis. This offline-LC-MS strategy has been confirmed to be useful for exploring the diversity of peptides from a spider venom (Escoubas, 2006). As shown in Figure 2D, a total of 164 mass peaks were detected and multiple mass peaks are present in

most HPLC fractions, indicating a single HPLC fractions actually contains various peptides. Approximately 90% of masses were localized in the mass range of 3000-4500 Da, showing most peptides from the venom might be composed of 27-40 amino acid residues, which are consistent with most spider peptide toxins identified so far.

3.2. Bioactivity Assays

The spider *H. venatoria* mainly feeds on insects. We determined the potential toxicity of the venom on *P. Americana* cockroaches. Intrathoracic administration of the venom immediately led to symptoms of envenomation including flaccid paralysis, lethargy and ataxia, while no such symptoms were observed in the cockroaches receiving injections of water. High doses of the venom could cause death. The LD₅₀ value was approximately 28.18 µg/g body weight (Fig. 3). This was much lower than those of other spider venoms, such as *H. huwenum* (300 µg/g) and *D. mizhoanus* (56.2 µg/g) (Li et al., 2014; Liang et al., 1992), showing the venom from the spider *H. venatoria* is more toxic than those spider venoms. In addition, the toxicity of the venom was also examined on mice. Intraperitoneal injection of 1mg venom/mouse (approximately 50 µg/g body weight, n=5) elicited no obvious toxic effects on mice. Because of the limit of the venom amount, no higher dose would not be applied. However, this preliminary data still highlighted that insects might more sensitive to the venom than mammals.

3.3. Effects of the venoms on VGSCs in insect DUM and DRG neurons.

VGSCs are essential in generating and propagating nerve impulses, placing them amongst the most widely targeted ion channels by toxins from venomous organisms (Bosmans and Swartz, 2010; Catterall, 2000). Most venomous animals have developed efficient toxins targeting VGSCs for defense or prey capture. The *H. venatoria* venom demonstrated neurotoxic and lethal activity on cockroaches, so we wondered whether the venom affected their VGSC currents. Using whole-cell patch clamp technique, the action of the venom was characterized on the VGSC currents of cockroach DUM neurons which is a useful model for insect VGSC research. Sodium currents were elicited using a 50 ms depolarization of -10 mV from a holding potential of -90 mV. The venom displayed strong inhibition on the VGSC currents in DUM neurons. The venom at the low concentration of 2 µg/mL could obviously suppress the peak currents, while 100 µg/mL venom led to complete inhibition of the peak currents (Fig. 4A; n = 5). From the dose-response curve (Fig. 4B; n = 5), the IC₅₀ value was estimated to be 6.25± 0.02 µg/mL. The effect of the venom (10µg/mL) on the current-voltage (I-V) relationship was determined as shown in Figure 4C. The venom reduced inward peak currents at all the depolarized voltages tested. It did not change the activation and reverse voltages, but it actually caused a positive shift of maximum activation voltage from 0 mV to +10 mV (Fig. 4C, n = 5). On the other hand, the effect of the venom on the mammalian VGSCs was also examined on rat DRG neurons (Fig 5). The venom exhibited much weaker inhibition on the VGSC currents in DRG neurons. Application of 10 µg/mL and 100 µg/mL venom only resulted in 10.2%

$\pm 2.1\%$ ($n = 5$) and $27.0\% \pm 3.7\%$ ($n = 5$) reduction of peak currents, respectively. These data indicate that the venom might contain potent and specific insect VGSC antagonists which are much less effective on mammalian VGSCs.

4. Conclusion

The development of novel insecticides from natural products is beneficial for insect pest control. Spider venoms containing diverse peptides have been considered as a rich source of insecticidal peptides. The spider *H. venatoria* is distributed worldwide and mainly feeds on insects. In the present study, we found this spider venom contains hundreds of peptides, most of which consist of 27-40 amino acid residues. The venom is lethal to cockroaches with anLD₅₀ value of 28.18 $\mu\text{g/g}$ body weight. This lethal effect might result from its potent inhibition on the VGSCs. Moreover, the inhibition of insect VGSCs by the venom was much stronger than that of mammal ones. Therefore, insecticidal peptides can be identified from the venom of the spider *H. venatoria* in the future. These insecticidal peptides might be insect-selective and have potential for novel insecticide development.

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References

- Attaran, A., Roberts, D.R., Curtis, C., Kilama, W.L., 2000. Balancing risks on the backs of the poor. *Nature medicine* 6, 729-731.
- Bass, C., Field, L.M., 2011. Gene amplification and insecticide resistance. *Pest management science* 67, 886-890.
- Bosmans, F., Swartz, K.J., 2010. Targeting voltage sensors in sodium channels with spider toxins. *Trends in pharmacological sciences* 31, 175-182.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 72, 248-254.
- Catterall, W.A., 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* 26, 13-25.
- Escoubas, P., 2006. Mass spectrometry in toxinology: a 21st-century technology for the study of biopolymers from venoms. *Toxicon : official journal of the International Society on Toxinology* 47, 609-613.
- Escoubas, P., Sollod, B., King, G.F., 2006. Venom landscapes: mining the complexity of spider venoms via a combined cDNA and mass spectrometric approach. *Toxicon : official journal of the International Society on Toxinology* 47, 650-663.
- Gunnell, D., Eddleston, M., Phillips, M.R., Konradsen, F., 2007. The global distribution of fatal pesticide self-poisoning: systematic review. *BMC public health* 7, 1.

- Gurevitz, M., Karbat, I., Cohen, L., Ilan, N., Kahn, R., Turkov, M., Stankiewicz, M., Stühmer, W., Dong, K., Gordon, D., 2007. The insecticidal potential of scorpion β -toxins. *Toxicon : official journal of the International Society on Toxinology* 49, 473-489.
- Herzig, V., Wood, D.L., Newell, F., Chaumeil, P.-A., Kaas, Q., Binford, G.J., Nicholson, G.M., Gorse, D., King, G.F., 2010. ArachnoServer 2.0, an updated online resource for spider toxin sequences and structures. *Nucleic acids research*, gkq1058.
- King, G.F., Gentz, M.C., Escoubas, P., Nicholson, G.M., 2008. A rational nomenclature for naming peptide toxins from spiders and other venomous animals. *Toxicon : official journal of the International Society on Toxinology* 52, 264-276.
- King, G.F., Hardy, M.C., 2013. Spider-venom peptides: structure, pharmacology, and potential for control of insect pests. *Annual review of entomology* 58, 475-496.
- Kuhn-Nentwig, L., Stöcklin, R., Nentwig, W., 2011. Venom composition and strategies in spiders: is everything possible? *Advances in Insect Physiology* 40, 1.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature* 227, 680-685.
- Li, J., Li, D., Zhang, F., Wang, H., Yu, H., Liu, Z., Liang, S., 2014. A comparative study of the molecular composition and electrophysiological activity of the venoms from two fishing spiders *Dolomedes mizhoanus* and *Dolomedes sulfurous*. *Toxicon : official journal of the International Society on Toxinology* 83, 35-42.
- Liang, S., 2008. Proteome and peptidome profiling of spider venoms. *Expert review of proteomics* 5, 731-746.
- Liang, S., Qin, Y., Zhang, D., Pan, X., Chen, X., Xie, J., 1992. Biological characterization of spider (*Selenocosmia huwena*) crude venom. *Zoological research/" Dong wu xue yan jiu" bian ji wei yuan hui bian ji* 14, 60-65.
- Oerke, E.-C., Dehne, H.-W., 2004. Safeguarding production—losses in major crops and the role of crop protection. *Crop protection* 23, 275-285.
- Pimentel, D., 2009. Pesticides and pest control, Integrated pest management: innovation-development process. Springer, pp. 83-87.
- Ramírez, M.J., 2014. The morphology and phylogeny of dionychan spiders (*Araneae: Araneomorphae*). *Bulletin of the american Museum of Natural History* 390, 1-374.
- Rash, L.D., Hodgson, W.C., 2002. Pharmacology and biochemistry of spider venoms. *Toxicon : official journal of the International Society on Toxinology* 40, 225-254.
- Saez, N.J., Senff, S., Jensen, J.E., Er, S.Y., Herzig, V., Rash, L.D., King, G.F., 2010. Spider-venom peptides as therapeutics. *Toxins* 2, 2851-2871.
- Sanguinetti, M.C., Johnson, J.H., Hammerland, L.G., Kelbaugh, P.R., Volkmann, R.A., Saccomano, N.A., Mueller, A.L., 1997. Heteropodatoxins: peptides isolated from spider venom that block Kv4. 2 potassium channels. *Molecular Pharmacology* 51, 491-498.
- Smith, J.J., Herzig, V., King, G.F., Alewood, P.F., 2013. The insecticidal potential of venom peptides. *Cellular and Molecular Life Sciences* 70, 3665-3693.
- Soderlund, D.M., Bloomquist, J.R., 1989. Neurotoxic actions of pyrethroid insecticides. *Annual review of entomology* 34, 77-96.
- Vassilevski, A., Kozlov, S., Grishin, E., 2009. Molecular diversity of spider venom. *Biochemistry (Moscow)* 74, 1505-1534.
- Wang, M., Rong, M., Xiao, Y., Liang, S., 2012. The effects of huwentoxin-I on the voltage-gated sodium

- channels of rat hippocampal and cockroach dorsal unpaired median neurons. *Peptides* 34, 19-25.
- Windley, M.J., Herzig, V., Dziemborowicz, S.A., Hardy, M.C., King, G.F., Nicholson, G.M., 2012. Spider-venom peptides as bioinsecticides. *Toxins* 4, 191-227.
- Xiao, Y.-C., Liang, S.-P., 2003. Inhibition of sodium channels in rat dorsal root ganglion neurons by hainantoxin-IV, a novel spider toxin. *ACTA BIOCHIMICA ET BIOPHYSICA SINICA-CHINESE EDITION* 35, 82-85.
- Yuan, C., Jin, Q., Tang, X., Hu, W., Cao, R., Yang, S., Xiong, J., Xie, C., Xie, J., Liang, S., 2007. Proteomic and peptidomic characterization of the venom from the Chinese bird spider, *Ornithoctonus huwena* Wang. *Journal of proteome research* 6, 2792-2801.

Figures and Legends:

Fig.1. The spider *Heteropoda venatoria*

Fig.2. (A).SDS-PAGE (15%) gel image of *H. venatoria* venom. (50 μ g) in lane 2, compared to the standard molecular weight ladder in lane 1. (B).HPLC profile of *H. venatoria* venom using a C18 column with varying percentage of acetonitrile as indicated on the right y-axis. (C).MALDI-TOF MS profile of *H. venatoria* venom. (D).Distribution of peptide molecular masses as a function of RP-HPLC elution time as determined by MALDI-TOF-MS analysis of individual RP-HPLC fractions of the venom

from *H. venatoria*.

Fig.3. Dose-response curve for intrathoracic injection of *H. venatoria* venom into American cockroaches . . The LD₅₀ is 28.18µg/g.

Fig.4. Effects of *H. venatoria* venom on whole-cell VGSCchannel currents in DUM neurons. (A). Na⁺ currents were induced by a 50 ms depolarized potential of -10 mV from a holding potential of -90 mV. Currents amplitude was blocked to $1.6 \pm 1.0\%$, $46.1 \pm 2.5\%$, $91.4 \pm 3.0\%$ of control (n=5) by 0.1 µg/mL, 2 µg/mL, and 100 µg/mL venom, respectively. (B). Concentration-dependent inhibition of VGSC currents in

DUM neurons by *H. venatoria* venom. Each data point (mean $\pm$ SE) shows the current as a fraction of the control current (n= 5). Data were fitted with a Boltzmann equation to yield an IC₅₀ value of 6.25 $\pm$ 0.02 μ g/mL. (c). Normalized currents induced before (control) and 5 min after 10 μ g/mL of *H. venatoria* venom application. Cells were held at -90 mV and Na⁺ currents were induced by 50 ms depolarizing steps to various potential ranging from -80 mV to +60 mV in 10 mV increments. Data are mean $\pm$ S.E.

Fig .5. Effects of *H. venatoria* venom on the Na⁺ currents in DRG neurons. Current traces were elicited by a 50 ms depolarizing potential of 0 mV from a holding potential of -80 mV every 5 s. Application of 10 μ g/mL or 100 μ g/mL of venom led to a reduction of Na⁺ currents of 10.2% $\pm$ 2.1% or 27.0% $\pm$ 3.7% of control (n = 5).

Table 1

Properties of *H. venatoria* spider venom.

Item	Determined Value
Wet venom yield	2-15 μ L/spider
Venom density	978 μ g/ μ L
Dried venom yield	78.23 μ g/ μ L
Protein content	32.34%
LD ₅₀ value in cockroach	28.18 μ g/g

Protein content is expressed based on dried venom; LD₅₀ values are expressed based on body weight.