

Study of some contention techniques for gathering tarantula venom

Studiul unor tehnici de contenție pentru recoltarea veninului de tarantulă

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Abstract

The study of various types of venom has become a priority in the last years for medical sciences and biology. Tarantula venoms represent a natural wealth of combinations of chemical compounds that attract more and more attention of pharmacologists. Over the last decade, these venoms have been studied to ultimately generate solutions: strong, selective bioactive products for medicine, but harvesting tarantula venom are a real challenge. In this respect, this initial present study aims to identify and analyze several methods of contention and anesthesia to allow operators an easier access to gathering the tarantula venom. Also due to scientific parsimony in relation to these venoms, data and specific uses in biology and medicine, we considered it necessary to present more in detail the main concerns of science on this topic at this time.

Rezumat

Studiul diverselor tipuri de venin a devenit, în ultimii ani, o prioritate pentru științele medicale și biologie. Veninurile de tarantulă reprezintă o bogăție naturală de combinații de compuși chimici care atrag tot mai mult atenția specialiștilor farmacologi. În ultimul deceniu aceste veninuri au fost studiate pentru a genera în final soluții: produse bioactive puternice, selective în folosul medicinei, dar recoltarea veninului de la tarantule este o reală provocare. În acest sens, studiul inițial de față își propune să identifice și să analizeze câteva metode de contenție și anestezie care să permită operatorilor un acces mai facil la recoltarea veninului de la tarantule. De asemenea datorită parcimoniei științifice în legătură cu datele despre veninul de tarantulă și utilizările specifice din biologie și medicină am considerat necesară o prezentare mai în detaliu a principalelor preocupări din știință pe acest topic, la această oră.

Objectives

We set the objectives:

- identifying viable methods of anesthesia and tarantula contention
- To carry out a study with data on the structure / utility of tarantula venom

Materials and methods

The study was conducted between April 2017 and May 2018 in the Pharmacology and Anesthesiology Laboratories at FMV Timișoara using these disciplines utilities.

Tarantulas

The individuals used in this experiment came from four different types of tarantula, all

of them obtained from their own terrariums in Timișoara where the tarantula females participating in this study were raised. For the accuracy of the results from the experiment, three replicas were performed with a repeat at one month to ensure the recovery of the venom reserve, using the same individuals as five, adult females.

Females have been treated gently throughout the study, none of which resulted in accidents or death. For the proper dosing of the narcotic we have chosen females of weights and close sizes / species.

Females of the tarantula in the study were ages 3 to 9 years, weighing between 17-24 g and 15-22 cm.

Tarantula feeding was done exclusively with a Blattodea beetle, the *Blaberidae* family, dubbed Guyana spotted roach or

Argentinian wood roach, used by breeders to feed tarantula once a week (Figure 4) [www.biolib.cz].

Growth and living conditions were uniform from the temperature point of the terrarium that ranged between 20-24°C for studied species but relative in relative humidity relative to:

- *Poecilotheria ornata* of 80%;
- *Eupalaestrus campestratus* of 70%,
- *Brachypelma klaasi* and
- *Lasiadora parahybana* 60-65%.



Figure 4. *Blaptica dubia* [www.biolib.cz].

From the point of view of skin shedding, none of the females in the study did not sneeze and was paired to not influence the effect of narcosis. Table 2 enlists the genus tarantula used in this study.

Table 2.

The species of tarantula used in the study (original photo Diana Cîmpian)

<p>1. <i>Poecilotheria ornata</i></p> <p>Medium weight: 17,3 g Medium length: 21,5 cm</p>	
<p>2. <i>Eupalaestrus campestratus</i></p> <p>Medium weight: 19,5 g Medium length: 15,3 cm</p>	
<p>3. <i>Brachypelma klaasi</i></p> <p>Medium weight: 21,5 g Medium length: 16,3 cm</p>	
<p>4. <i>Lasiadora parahybana</i></p> <p>Medium weight: 24,0 g Medium length: 22,3 cm</p>	

Methodology of harvesting and analyzing venom

To harvest the tarantula venom and avoid the risk of bite, they must be anesthetized, because their safe contention is almost impossible when they are vigilant.

In the methodology of testing the tarantula venom, four steps are being observed regarding the handling of tarantula and one dedicated to biochemical analysis, respectively, as follows:

- tarantula anesthesia,
- contention of anesthetized tarantula,
- application of electric stimuli on chelicerae,
- harvesting venom,
- biochemical analysis of venom.

Phase I: Tarantula anesthesia

Anesthesia (Chamber) Device

For anesthesia, an anesthetic device (a chamber) was made of a plastic food container with a sealed cap, fitted with two attachments to the anesthesia systems for the access and evacuation of anesthetic vapors), imagined by Dr Larisa Schuszler, head of anesthesiology discipline at FMV Timisoara (Figure 5).



Figure 5. Device (chamber) for anesthesia and coupling to the dosing device

Anesthetic and dosage

The active substance used was an indigenous product, inhaled isoflurane (Anesteran, Rompharm Company Romania) (Figure 6), a general anesthetic that is considered by most authors as a suitable substance for insect anesthesia / narcosis also [www.rompharm.ro].

The product is a colorless liquid with a clear appearance for inhalation vapors.

The *induction and maintenance* of anesthesia with 5% isoflurane (Anesteran) was performed in an oxygen flow of 500 ml / L / minute by means of an anesthesia machine through a tube connected to the anesthetic device (camera), following the following:

- the anesthetic has been uniformly and equally applied to all specimens;
- each tarantula was individually anesthetized;
- *the maintenance time* in the "Anesthesia Chamber" was 10 minutes for all females.



Figura 6. Volatile solution of isoflurane (Anesteran, Rompharm Company Romania) and dosage of anesthetic at 5%.

**Phase II:
Manual contention**

In all cases, complete narcosis only occurred two minutes after the induction of the anesthetic and it lasted long enough for tarantulas to be susceptible to electrical stimulation. For arboreal tarantulas, which have the flattened body, the preferred contentious mode is between the opposing fingers in the longitudinal plane and in the case of the old world tarantulas, the contention is between the fingers in the sagittal plane (figure 7).



Figure 7. Anesthetized tarantula and types of contention

**Phase III:
application of electrical stimuli on chelicers and harvesting of venom**

Applying electrical stimuli

For our study we tested different current sources with voltage ranging from 4.75 to 15

volts (Figure 8) with amperage values ranging from 0.55 to 20 amperes as follows:

- 9 volt battery,
- 12 volt battery,
- 4,75 volt - 0,55 Amp. battery,
- 5 volt - 1.0 Amp, battery
- 5.3 volt - 2 Amp, battery
- 15 volt - 20 Amp. power rectifier.



Figure 8. Electric power sources used in the experiment

The harvested venom was deposited in sterile containers. For this study we used the microcentrifuge tubes (Figure 8).

Venom's gathering

The tarantulas were manually constrained to have better control for their safety (figure 9).

After fixing the chelicers in the micro centrifuge tube, we applied the electric stimuli on the chelicers in the order of increasing the voltage from: 4,5; 5; 5.3; 9; 12 and 15 volts (Figure 9).

In general, the restoration of the stock of venom to the tarantula is done 7-14 days, but for proper harvesting it is advisable to stimulate at least one month resting interval

Vennom's conservation

Venom obtained by electrical stimulation was solubilized in deionized water containing 12% trifluoroacetic acid (TFA) and centrifuged at 10000 rpm for 10 min. The solubilized

supernatant in this way was frozen, lyophilized and stored at -20 ° C. The dry weight of the venom is determined by analytical weighing of high precision.

Phase IV: Biochemical analysis of venom

Sample preparation

For chromatographic analysis aliquots of 5 mg of dry venom were solubilized in deionized water, centrifuged at 10000 rpm for 10 min, after which the supernatant was subjected to high performance liquid chromatography (HPLC). The eluted fractions at 1.5 ml / min were collected individually and manually, vacuum-dried and stored at -20 ° C until use.

Used columns

Were C18 reverse phase semi preparative (ie Jupiter 5 mm, 300 A, 250-10 mm) using a linear gradient composed of the initial solution A (0.12% TFA) to 60% and solution B (0, 10% TFA in acetonitrile - ACN) for 60 minutes after 10 minutes initially at 0% solution B. Wavelengths of 216 and 230 nm are used for detection.

To obtain low molecular weight fraction (LMMF) and protein fraction (PF) for the evaluation of venom activity, the fractions eluting from 0 to 35% of solution A and from 35 to 74% of solution B were collected separately.

After removing the solvent, the LMMF and PF venoms were quantified by measuring the dry weight at the high precision analytical balance and stored at -20 ° C until use. The molecular masses of the chromatographic fractions of the tarantula venom can be carried out on a mass spectrometer such as UltraFlexIII MALDI-TOF / TOF.

The samples are reconstituted in deionized water at varying and dissolved concentrations (1: 3, v: v) in a solution of α -cyano-4-hydroxycinnamic acid matrix (α -cyano-4-hydroxycinnamic acid at 5 mg / trifluoroacetic acid, 5: 4: 1, v: v: v) punctuated

in triplicate on a sample plate and allowed to dry at room temperature.

MS spectra will be obtained both in reflected and linear mode. Calibration of the system is performed using a mixture of the peptide calibration standard and the protein I calibration standard for mass spectrometry, after which the spectra are processed using the MassLynx 3.5 and / or Flex Analysis 3.3 analyzers [Bohlen et al., 2010].

Results and discussions

From our observations, the anesthetic with the local product Anestran (Rompharm Company Romania) volatile solution proved to be a good choice for tarantulas anesthesia, the dosage at the 5% concentration being considered to be optimal.

Being elected in equal batches as size and weight, in narcotic phase we did not noticed time differences between specimens / species in relation to the induction phase of narcosis.

In all specimens, we noticed that full narcosis was installed very quickly, or between 100 and 120 seconds (Figure 9).

The fastest narcosis was installed in the species *Eupalaestrus campestratus* and at the latest in the species *Lasiadora parahybana*.

Regarding the duration of narcosis, it ranged from 5 to 7 minutes, depending on individual and species, so that the longer narcosis period was observed in the species *Poecilotheria ornata* and *Eupalaestrus campestratus*, while longer periods were identified at the species *Brachypelma klaasi* and *Lasiadora parahybana*.

The duration of narcosis was considered by us to be sufficient for the electrical stimulation phase.

Regarding the electric stimulation stage, we noticed that in all cases the venom emission was possible by the electric shock of the chelicerae.

Of all the voltages used we recommend the use of voltages of 12 or 15 V and 25-85 Hz, voltages under this voltage, respectively: 4,5; 5; 5.3 and 9 V followed by excitement of the venom glands.



The value of 15 volts leads to the correlation of the venous glands, so it is in our opinion an optimal value, especially if they are applied to moistened chelicerae with physiological saline or any saline solution, which will amplify the stimulation and the connection of glands to the venom (figure 9).

As the fresh venom is transparent and viscous consistency. The amount of venom obtained after electrical stimulation was small in the form of drops, the mass of a venom discharge being approximately 10µg (figure 9).





Figure 9. Phases of narcosis, contention and harvesting of tarantula venom

Discutions

Applications of venom from various tarantula species in biology and medicine

In the last decade, the implementation of new biochemical investigation methods has boosted the complex structure of venom [Dumitrescu and Cristina, 2015].

With deciphering the types of proteins associated with various types of venom, researchers began to test venom (in their entirety or just a few isolated venom sequences) in the therapeutics of various morbid entities or in biology [Kocsis and Cristina, 2018].

In this respect, venoms from various tarantula species have been studied biochemically and successfully tested in medicine or biology.

Of these, already successfully researched the species's venom:

- *Psalmopoeus cambridgei*
- *Selenotypus plumipes*
- *Selenocosmia jiafu*
- *Ornithoctonus huwena*
- *Tarantula cubensis*

- *Tarantula de Canton*
- *Grammostola spatulata*
- *Acanthoscurria paulensis*

Psalmopoeus cambridgei ("Trinidad Chevron")

A peptide extracted from the South American tarantula venom *Psalmopoeus cambridgei* is effective against pain through ASIC1a channels and opioid mechanisms.

Psalmotoxin 1, a peptide extracted from tarantula *Psalmopoeus cambridgei*, has very strong analgesic properties against thermal, mechanical, chemical, inflammatory and neuropathic pain in rodents.

It exerts its action by blocking the ionic channel sensitive to 1a acids, and this blockade results in an activation of the endogenous encephaline pathway.

The analgesic properties of the peptide are suppressed by the opiate receptor antagonists *l* and *d* and are lost in the Penk1 - / - mice [Mazzuca et al., 2007].

At this tarantula, for the first time, the three ICK peptides (referred to as *vanilotoxins VaTx1, 2 and 3*) have been discovered to activate TRPV1 producing a strong inflammatory pain [Milescu et al., 2009].

Vanilotoxins show a similarity to the hanatoxin sequences, and VaTx1 and VaTx2 inhibit Kv2.1, thus supporting speculation that TRP and variable-voltage channels resemble each other in terms of structure and / or closure mechanisms.

Vanilotoxins are excellent pharmacological structures, but rapid dissociation rates are technically limiting their usefulness as biochemical tools for studying the structure of the TRP channel [Kuhn-Nentwig et al., 2011].

Selenotypus plumipes

Australian researchers have identified the first bio-insecticide in *Selenotypus plumipes* tarantula.

They purified the venom derived in different fractions and offered them as food for termites, flour worms, and cotton worms.

The results revealed the lethal activity of the OAIP-1 (Orally Active Insecticidal Peptide) that killed these insects in two days.

To our knowledge, OAIP-1 is different from other insecticidal bio-structures because it is potent, synergistic with some insecticides and can be obtained by recombination.

The structure of this peptide is stable at high temperatures and at a basic pH and can be used as a foliar spray.

OAIP-1 is a powerful insect neurotoxic, the required dose being much less than synthetic insecticides [Kuhn-Nentwig et al., 2011].

Selenocosmia jiafu

It is a medium-sized tarantula and an attractive source of venom because it can be grown in captivity and produces large amounts of venom.

The Selenocosmia venom contains mainly neurotoxins that act on voltage-dependent ion channels from the roots of the dorsal nerve neurons in rats.

Venom analysis by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) and MALDI-TOF-MS (Matrix Assisted Laser Desorption / Ionization Time of Flight Mass Spectrometry) revealed that venom contains hundreds of peptides with a predominant mass of 3000-4500 Da.

Venin has inhibitory effects on tetrodotoxin-resistant (TTX-R) Na⁺ and T-Ca²⁺ types suggesting the presence of antagonists for both types of channels.

Experimental intra-abdominal injection with venom had severe toxic effects on beetles including posterior paralysis.

The LD₅₀ (lethal dose) determined was 84.24 µg/g of beet weight.

However, no visible symptoms or behavioral changes were observed in mice after intraperitoneal injection with venom doses up to 10 mg / kg body weight.

At a dose of 20 mg / kg body weight, mice showed poor poisoning symptoms, which decreased after 30 minutes.

Only at the dose of 46.30 mg / kg body weight the mice showed obvious signs of

intoxication and died 24 minutes after the injection of the venom [Escoubas and Rash, 2004].

Ornithoctonus huwena (Chinese bird spider)

It is a particularly aggressive old world tarantula, living in underground shelters in the tropical regions of southern China and Vietnam.

Generally, the bite of *Ornithoctonus huwena* is not lethal to humans, but can cause major pain and inflammation.

The toxin originating from this tarantula is a specific and irreversible TRPV1 agonist.

Structurally, the toxin contains two ICK domains folded independently with antibody-like bivalence, resulting in an extremely high avidity for the multimeric channel target, making it a biochemical instrument for analyzing the function of the TRP channel.

This toxin binds and captures TRPV1 in open state by associating with the channel pore forming region rather than the equivalent region of the voltage sensor near the S3 and S4 spirals [Gabbiness, 1979].

These observations support the critical role of vanillotoxins for the field of pore formation in the TRP channel and suggest that conformational changes in the outer pores may be more important than those previously evaluated [Gultiken et al., 2015].

As in the case of Psalmopoeus cambridge, the crude venom activates recombinant TRPV1, the authors suggest that the venom contains one or more peptide toxins targeting nociceptors as part of the chemical defense strategy [Liang, 2004].

The major active components of this venom were revealed using calcium ion imaging as a functional reading method (figure 10).

Several species of tarantula target TRPV1 channels.

Many peptide toxins, including vanillotoxins VaTx1 and VaTx2, are known to target multiple channel subtypes.

To evaluate the specificity of *O. huwena* purified toxin, the effect of a relatively high

dose (2 mM) on TRP channels (TRPV2, TRPV3, TRPV4, TRPA1 and TRPM8), legated (5-HT_{3R-A} and P2X₂) and dependent voltage channels (Kv1.2, Kv2.1 and Kv4.3, CaV1.2, CaV3.3 and NaV1.7, respectively), many of which are expressed in sensory neurons. When DkTx was applied to oocytes or HEK293 cells, those expressing any of these channels no effect was observed.

In accordance with this apparent selectivity for TRPV1, imaging experiments with calcium ions and trigeminal neurons cultivars revealed the influx of toxin-evoked calcium into a subset of neurons corresponding to capsaicin-sensitive groups

In addition, this response was absent in cultures derived from mice deficient in TRPV1 (Figure 10) [Bohlen et al., 2010; Gultiken et al., 2015].

The absorption and migration profile of *O. huwena* toxin on inverse phase matrices suggested that the active component is hydrophobic and peptide in nature.

Considering its relatively large size (monoisotopic mass of 8521.9 Da) and relative palatability of the venom, so far only partial toxin sequences have been obtained by sequencing de novo peptides.

To circumvent this problem, the researchers prepared total RNA from the *O. huwena* venom glands and used the material to clone complementary DNAs (cDNAs), which encode mature toxins.

The sequences obtained were consistent with the mass of the full-length toxins and that of the proteolytic-derived fragments.

Furthermore, the native toxin contained a C-terminal amidated arginine, consistent with the fact that the cDNA sequence predicts the transfer of an amide group from a glycine residue to the $n + 1$ position of the precursor peptide [Bohlen et al., 2010].

Although the *O. huwena* toxin contains a pattern of cysteine residues conforming to the observed ICK model and vanillotoxins, the toxin has a small sequential similarity with vanillotoxins, suggesting that *P. cambridgei* and *O. huwena* have developed TRPV1 agonists independently through a lengthy process of convergent evolution.

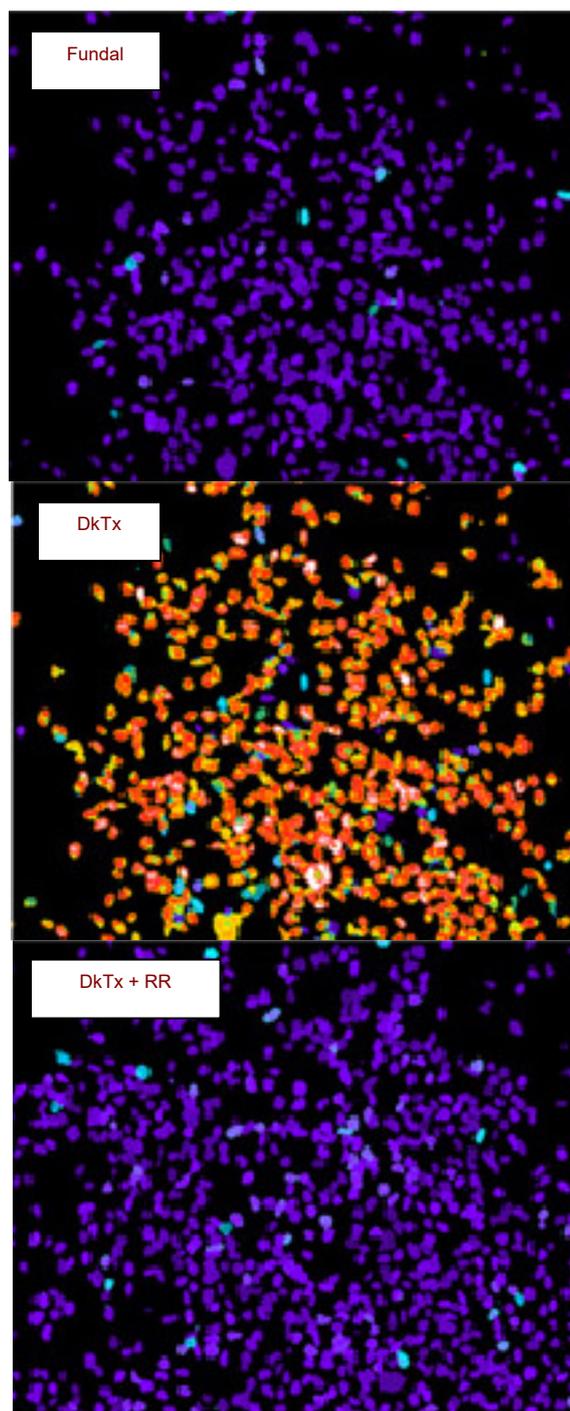


Figure 10. Influence of DkTx toxins on HEK293 cells expressing the rat TRPV1 channel [Bohlen et al., 2010].

Purified DkTx toxin evokes significant increase in calcium in HEK293 cells that express the rat TRPV1 channel.

Applying the 10 mM ruthenium red (RR) solution, which is a non-selective porous TRP channel blocker, will inhibit toxin evoked responses.

The purple color indicates low cytoplasmic calcium and orange indicates increased calcium.

It is also known that structurally, *O. huwena* toxin is approximately twice as high as vanillotoxins and consists of two repeats of the head-to-tail ICK unit separated by a short linker (Figure 11).

DkTx (K1)	DCAKEGEVCSWGKCCDLDNFYCPMEFIPHCCKYKPYVPVTT-
DkTx (K2)	-NCAKEGEVCGWQSKCCHGLD..CPLAFIPYCEKYR
VaTx1	SECRWFMGGCDSTLDCCKHLS..CKMGLY.YCAWDGTF
VaTx2	GACRWFLGGCKSTSDCCEHLS..CKMGLD.YCAWDGTF
VaTx3	ECRWYLGCKEDSECCEHLQ..CHSYWE.WCLWDGSF
HaTx	ECRYLFGGCKTTSDCCKHLG..CKFRDK.YCAWDFTFS

Figure 11. DkTx is a member of the ICK peptide family [Bohlen et al., 2010].

The toxin from *O. huwena* tarantula venom is a bivalent peptide ICK (*cysteine knot inhibitor*).

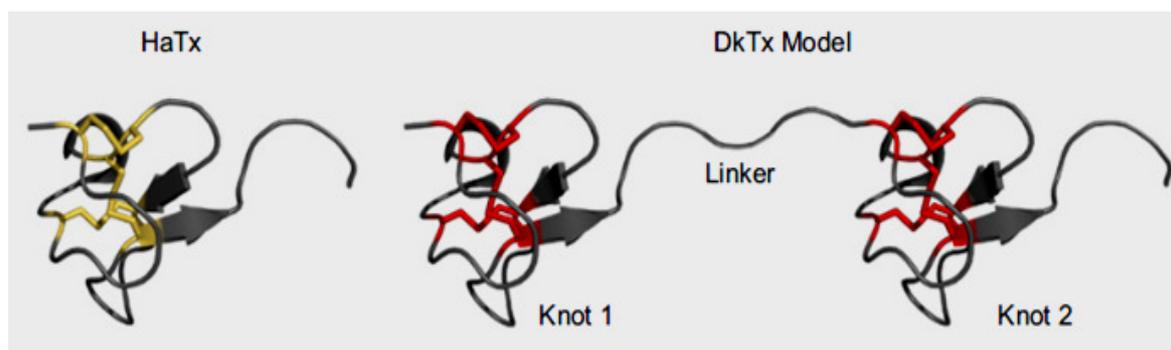


Figure 12. Bivariate structure of DkTx (Knot 1 and Knot 2) [Bohlen et al., 2010].

DkTx is a member of the ICK peptide family. In addition to the highly conserved cysteine residue structure (highlighted in yellow), DkTx shows no other apparent sequence similarities with other ICK-like peptides, including vanillotoxins (VaTx1-3) or hanatoxin (HaTx) [Bohlen et al., 2010].

Tarantula cubensis (Canton tarantula) (TCE)

The *Tarantula cubensis* venom extract modifies the degree of apoptosis and mitosis in mammary adenocarcinoma in the bitch.

In order to understand the effect of *Canton tarantula* Venom (TCE) extract on

ICK units adopt a compact and rigid characteristic structure and therefore *O. huwena* toxin is considered to form two independently folded domains connected by a tapered band (Figure 12).

Because this *O. huwena* peptide shows an example of a toxin containing repetitively repeated ICK structures, it was called the "double knot" or DkTx (Figure 12) [Bohlen et al., 2010].

DkTx consists of two ICK lobes (Knot 1 and Knot 2) separated by a short binding region.

The structure of the HaTx nuclear magnetic resonance solution served as a template for a hypothetical model of DkTx, showing the disulphons conserved in yellow for HaTx and red for DkTx (Figure 12) [Bohlen et al., 2010].

breast tumor tissue, 13 clinical cases of canine breast adenocarcinoma [Albay et al., 2010].

Biopsies were taken from the tumors prior to initiation of TCE treatment, and subcutaneous TCE injections were performed three times at weekly intervals of 3 ml / batch, and then at 7-10 days after the third injection, the masses tumors were extirpated by full unilateral mastectomy technique and pre- and post-treatment tumor formations were evaluated immune-histo-chemically.

The results revealed that the expression of B cell lymphoma (Bcl-2) was significantly higher in pretreatment compared to treated tissues ($p < 0.01$), while Ki-67 expression was lower in tissues evaluated after treatment ($p < 0.01$).

No significant differences were observed between fibroblast growth factors or vascular endothelial growth factor expression between pre- and post-treatment tissues ($p > 0.05$), and the apoptotic index was low before treatment and increased during treatment.

These results clearly suggest that TCE might be effective in controlling the growth of canine mammary adenocarcinoma by regulating apoptosis [Albay et al., 2010].

It has been observed that *Tarantula cubensis* extract is effectively used as a treatment in oral lesions in bovine animals with blue-tongue disease.

According to the hematological results, a marked leukocytosis due to lymphocytosis was observed, while erythrocytes, hematocrit and hemoglobin values were within normal limits.

After 24 hours, the mean leukocyte values were 22.15 ± 4.25 and 17.11 ± 2.01 for the control group and for the treated groups, respectively.

The number of leukocytes decreased in the treatment group after 24 hours compared to the control group.

The mean heart rate in the control and treatment groups during the study was not significantly different.

A significant decrease in rectal temperature was observed 24 hours after treatment in the treatment group compared to the control group [Albay et al., 2010; Gultiken et al., 2015].

Rapid healing was observed in oral mucosal lesions 24 hours after treatment in the treatment group versus the control group.

A statistically significant difference ($p < 0.05$) was observed between the median of oral lesions in control and treatment groups after 24 hours.

On the tenth day of treatment, all bovine animals in the treatment group were recovered [Albay et al., 2010; Gultiken et al., 2015].

Grammostola spatulata

A peptide from the tarantula venom of Chilean *Grammostola* can change the heart rate.

Atrial fibrillation is the most common sustained cardiac arrhythmia that occurs in humans and is secondary to valve disease, hypertension, or heart failure.

This it is often associated with the passive stretching of the atria chamber resulting from hemodynamic or mechanical dysfunction of the heart.

Research has shown that dilatation-enhanced atrial fibrillation in the heart of the rabbit can be inhibited by blocking stretch-activated ion channels with a specific peptide from tarantula venom without altering the potential for resting action.

Their findings open a window on cardiac arrhythmogenesis and pave the way for the development of a new class of drugs [Dobson, 2001].

GsMtx-4 is a small peptide (relative molecular weight, 4K) found in spatula *Grammostola* venom (figure 13).

This it specifically blocks cationic SACs in astrocytes and inhibits volume-activated currents in both adult astrocytes and cardiocytes. As a member of the "node-cysteine" neuro-inhibitory family, GsMtx-4 has a binding affinity of about 500 nM for SAC in astrocytes.

The specificity of its action is indicated by its lack of effect on the resting properties of rabbit ventricle cells and rat astrocytes [Caldwell et al., 1998].

At 4 nM (approximately 20 times the dose applied to suppress fibrillation), GsMtx-4 had no measurable effect on the potential of isolated atrial cell [Dobson, 2001].

The incidence of the camera increased the incidence and duration of fibrillation (figure 13).

At pressures of over 12 cm H₂O, the probability of sustained fibrillation (for more than 60 seconds) approached the unit.

The infusion of 170 nM GsMtx-4 suppressed both the incidence and duration of fibrillation in all hearts (n410).

At pressures below 17.5 cm H₂O, sustained fibrillation was completely inhibited in all preparations (data not shown).

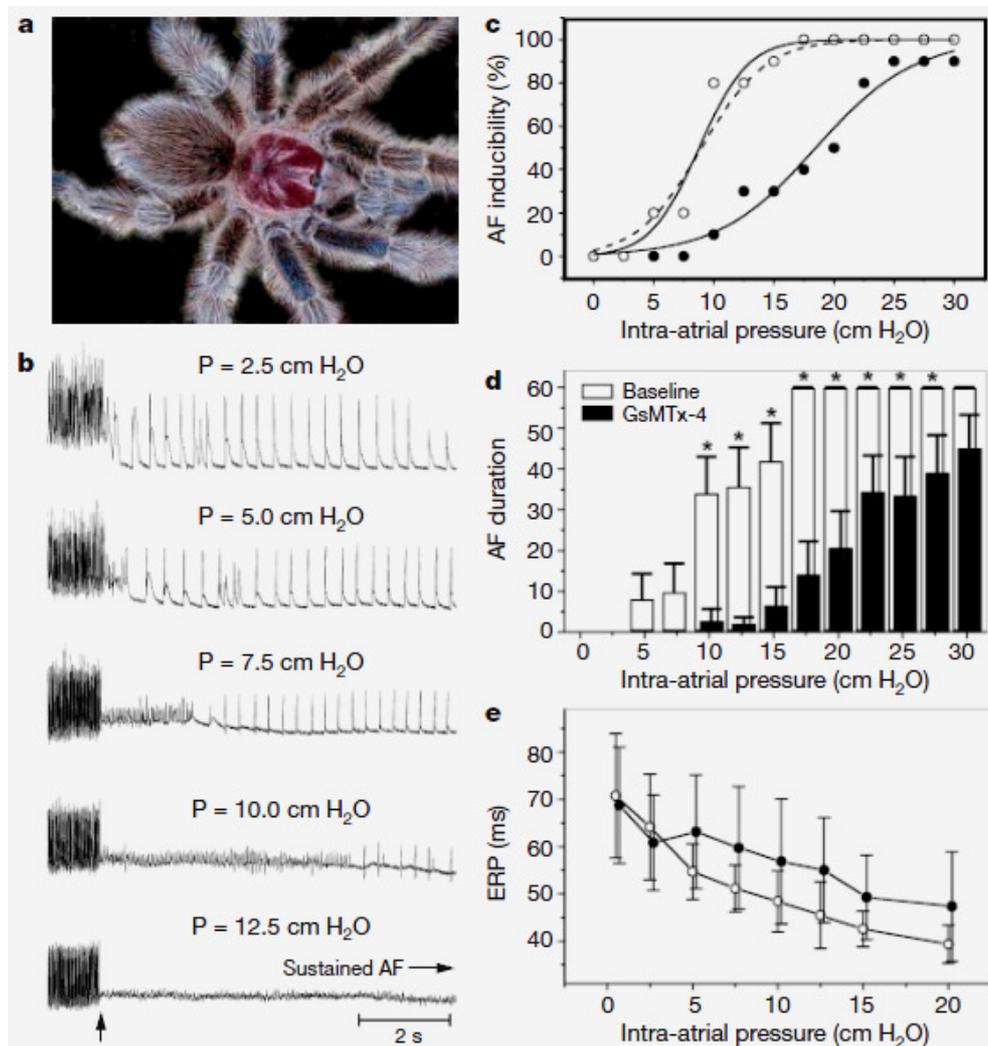
The gadolinium ion (Gd³⁺), a non-selective SAC inhibitor, can also suppress

stretch-induced cardiac fibrillation [Bode et al., 2000; Caldwell et al., 1998] but its lack of specificity and inapplicability under physiological conditions [Suchyna et al., 2000] limits its testing on the SAC.

In spite of their different chemical structures, both GsMTx-4 and Gd3 + suppress fibrillation in a similar manner without altering the dependence on stretching of the actual refractory period (figure 13), probably because both reagents have a high positive charge density.

The ant fibrillatory effect we see here cannot be determined by a change in the density of the surface load, as this would change the shape of the potential for action.

A different mechanism is also indicated by persistence of leakage-induced leakage of the refractory period while fibrillation is inhibited. Selective SACs K, [Kim, 1992] that are resistant to Gd3 [Nazir et al., 1996] and possibly to GsMtx-4, may also act to shorten the stretching potential (Figure 13).



Inhibition of atrial fibrillation by GsMtx-4 during stretching. **a.** *Spatula grammostola* whose venom is the source of the inhibitory peptide. **b.** Bipolar atrial electrodes showing an increase in pressure atrial fibrillation (AF), sustained at 12.5 cm H₂O. The probability of inducing AF was increased by stimulating the heart with a short high frequency stimulation period before each measurement (arrow). **c.** AF induction that lasts more than 2 s: cycles, control; in the filled circles, in the presence of 170 nM GsMtx-4. The broken line indicates the response after a 20 minute washes. **d.** AF duration (n47) by pressure (mean±s.e.). GsMtx-4 (170 nM) reduced the mean spontaneous recovery time from AF (P * 0.05). **e.** GsMtx-4 did not block the induced lengthening of the refractory period (n410).

Tension sensitivity is not unique to the atrium, so GsMtx-4 should act similarly in all rooms [Bode și col., 2001].

As an investigative tool, this peptide should be useful for studying mechanical transduction in the molecules and the entire body. It is believed that GsMtx-4 could be the first of a new class of antiarrhythmic agents to be directed against the causes rather than the symptoms of fibrillation [Dobson, 2001].

Acanthoscurria paulensis (Brazilian spider)

The fractionation of *A. paulensis* venom by RP-HPLC gave a total of 60 chromatographic fractions (Figure 14).

Of these, 97 distinct components were identified by MALDI-TOF MS analysis, with molecular weights ranging from m/z 601.4 to 21,932.3 [Mourão et al., 2013].

Molecular mass analysis obtained by mapping *A. paulensis* venom mass spectrometry reveals the presence of three major molecular component groups, with 30% of the components between 500 and 1999 Da and 38% the range of 3500 and 5999 Da.

A third group distributes from 6500 to 7999 Da, with about 21%.

Low molecular weight compounds (<1 kDa) are present in most fractions analyzed.

The m/z 601.4 and 729.6 ions were found in abundance in the most hydrophilic but found to be spread across several elution fractions (figure 14).

Peptides with a molecular weight greater than 2000 Da were observed only in phase 34 (ACN 37%), both in modes (500-6000 Da) and linear (3.5-15 kDa and 10-40 kDa).

Since, for the evaluation of cardio toxicity, fractions that eluted from 0 to 35% ACN and from 35 to 74% ACN were collected separately and were called the low molecular weight fraction (LMMF) and the protein fraction (PF) [Mourão și col., 2013].

The frequency of the molecular weight of the chromatographic fractions obtained from the *A. paulensis* venom revealed that 97 different molecular weights were distributed in 500 Da classes with a range of 500 to 25000 Da (figure 15).

Data were obtained by MALDI-TOF / TOF MS chromatographic analysis, which functions in positive and linear positive modes, with α -cyano-4-hydroxycinnamic acid as the matrix solution [Mourão și col., 2013].

The distribution of the molecular mass of the compounds present in the *A. paulensis* venom according to their HPLC elution profile is shown in figure 16.

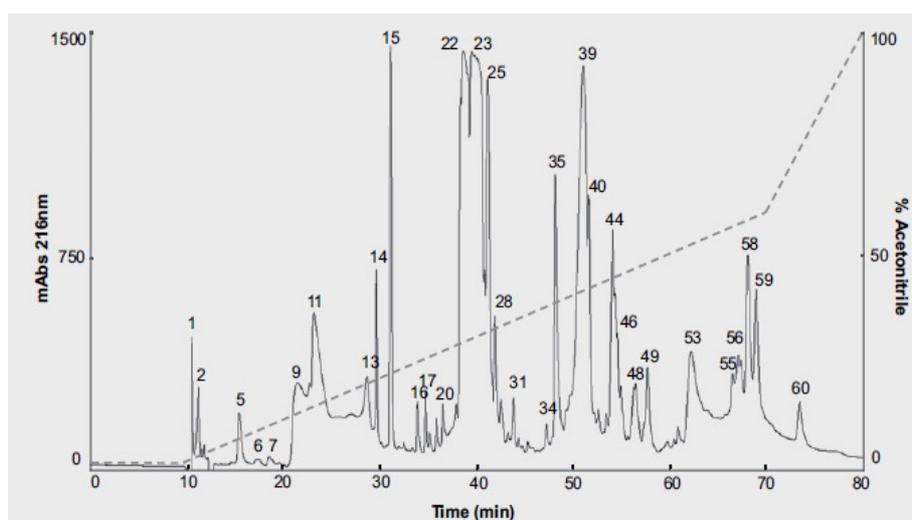


Figure 14. Chromatographic profile of the venom of *Acanthoscurria paulensis* [Mourão et al., 2013].

Chromatographic profile of 5.0 mg of soluble venom *Acanthoscurria paulensis*. RP-HPLC fractionation in a C18 semi-preparative column (Phenomenex) using an acetonitrile gradient (ACN) which is the discontinuous line at a flow rate of 1.5 ml / min and absorption at 216 nm. The main fractions are numbered.

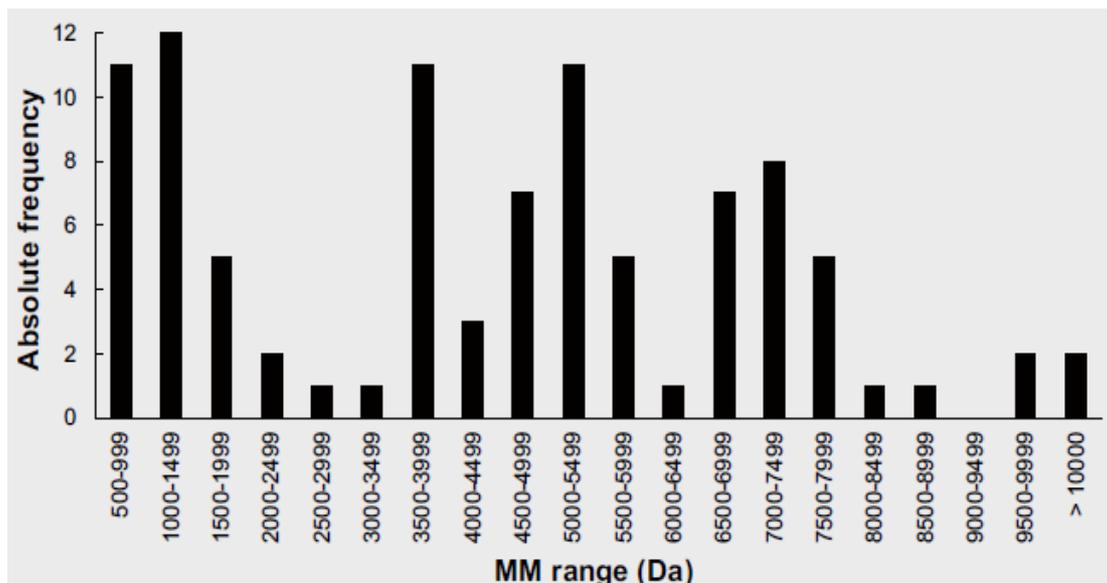


Figure 15. Frequency of the 97 molecular masses of *A. paulensis* venom [Mourão et al., 2013].

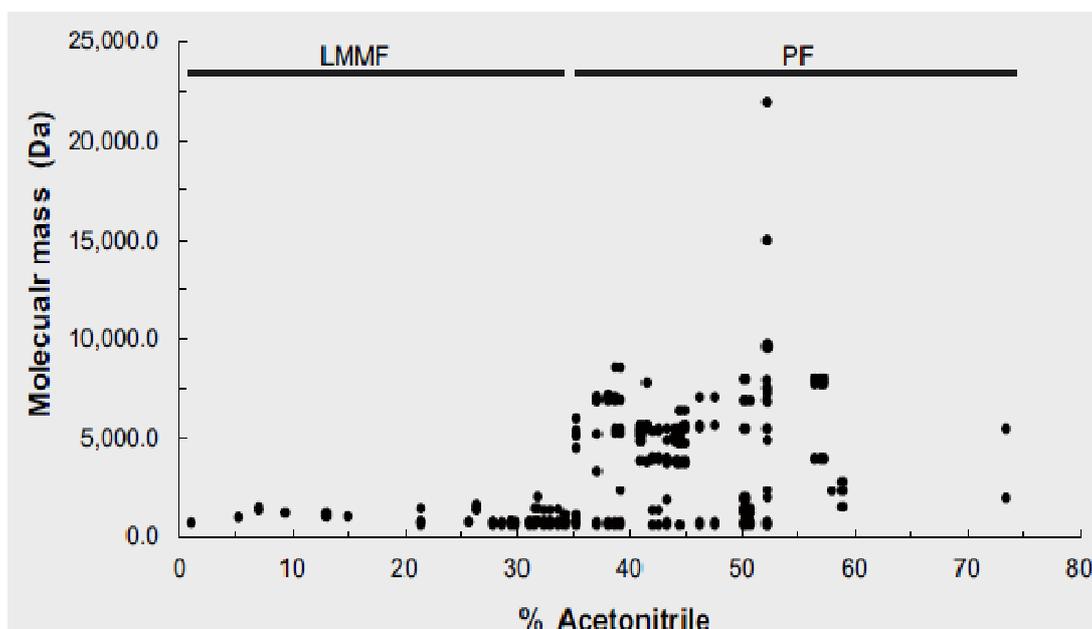


Figure 16. Distribution of the molecular weight of the compounds present in the *A. paulensis* venom depending on their elution profile [Mourão et al., 2013].

Chromatographic fractions were obtained by RP-HPLC as mentioned above.

Data obtained by MALDI-TOF / TOF MS analysis which operates in positive and linear modes, from 500 to 25,000 Da, with -Cyano-4-hydroxycinnamic acid as the matrix solution.

Bars refer to low molecular weight fraction (LMMF) and protein fraction (PF) fractions, both fractions separately collected and used in the ventricular band test [Mourão și col., 2013].

Conclusions

- The study of tarantula has become an important field of research for medicine because of the diversity of species and the multitude of the protein structure of their venom (90-110) with a role already known in medicine.
- The induction of narcosis with isoflurane is a viable option for tarantula, it was done after a short induction period of 100-120 seconds.

- The period during which the tarantula was anesthetized ranged from 5 to 7 minutes depending on the species. The fastest narcosis was installed in the species *Eupalaestrus campestratus* and at the latest in the species *Lasiodora parahybana*.
- A good electrical stimulation of the chelators to be followed by venom secretion is done at 12 or 15 volts, lower voltages not effecting. Moisture of saline chelicerae has favored transmission of electrical stimuli and venom relapse.
- Fresh venom gathering is carefully done in harvesting tubes with a venous outlet of about 10 µg.
- Pentru conservare condițiile sunt identice cu alte veninuri nefiind necesare măsuri / manopere dificile. Veninul liofilizat și păstrat în condiții corespunzătoare este bioactiv pentru perioade foarte lungi de timp.
- In recent years, the new investigative methods implemented in biochemistry have led to a significant increase in knowledge of the complex structure of tarantula venom. Successful researches on the venom of the species are known: *P. cambridgei*, *O. huwena*, *S. plumipes*, *S. jiafu*, *O. huwena*, *T. cubensis*, *Canton tarantula*, *G. spatulata* or *A. paulensis*

References

- Albay, M.K., Şahinduran, Ş., Kale, M., Karakurum, M.Ç., Sezer, K. (2010).** Influence of *Tarantula cubensis* Extract on the Treatment of the Oral Lesions in Cattle with Bluetongue Disease. *Kafkas Univ Vet Fak Derg*, 16(4):593-596.
- Bode, F., Katchman, A., Woosley, R.L., Franz, M.R. (2000).** Gadolinium decreases stretch-induced vulnerability to atrial fibrillation. *Circulation*, 101:2200–2205.
- Bode, F., Sachs, F., Franz, M.R. (2001).** Tarantula peptide inhibits atrial fibrillation. *Nature*, 409(6816):35-36.
- Bohlen, C.J., Priel, A., Zhou, S., King, D., Siemens, J., Julius, D. (2010).** A bivalent tarantula toxin activates the capsaicin receptor, TRPV1, by targeting the outer pore domain. *Cell*, 141(5):834-845.
- Caldwell, R.A., Clemo, H.F., Baumgarten, C.M. (1998).** Using gadolinium to identify stretch-activated channels: technical considerations. *Am J Physiol*, 275(2 Pt1):C619–C621.
- Dobson, R. (2001).** Spider venom may prevent atrial fibrillation. *West J Med*, 174(3):164.
- Dumitrescu, E., Cristina, RT (2015).** Elemente de terapie alternativă și complementară în medicina veterinară. Solness Timisoara, pp: 47-49.
- Escoubas, P., Rash, L. (2004).** Tarantulas: eight-legged pharmacists and combinatorial chemists. *Toxicon*, 43(5): 555–574.
- Gabbiness, S.G.T. (1979).** Comparison of venom components from four tarantula species. *Thesis*, Oklahoma State Univ., USA.
- Gultiken, N., Guvenc, T., Kaya, D., Agaoglu, A.R., Ay, S.S., Kucukaslan, I., Emre, B., Findik, M., Schäfer-Somi, S., Aslan, S. (2015). *Tarantula cubensis* extract alters the degree of apoptosis and mitosis in canine mammary adenocarcinomas. *J Vet Sci*, 16(2):213-219.
- Kim, D.J. (1992).** A mechanosensitive K⁺ channel in heart cells. Activation by arachidonic acid. *Gen Physiol*, 100(6):1021-1040.
- Kocsis, R., Cristina, RT.** Despre reptile – Un mic ghid pentru cei pasionați. Waldpress Timisoara, pp: 181.
- Kuhn-Nentwig, L., Reto Stöcklin, R., Nentwig, W. (2011).** Venom Composition and Strategies in Spiders: Is Everything Possible? In Jérôme Casas, editor: *Advances in Insect Physiology*, Vol. 60, Academic Press, Elsevier Ltd., pp. 1-86. ISBN: 978-0-12-387668-3.
- Liang, S. (2004).** An overview of peptide toxins from the venom of the Chinese bird spider *Selenocosmia huwena* Wang [*Ornithoctonus huwena* (Wang)]. *Toxicon*, 43(5):575–585.
- Mazucca, M., Heurteaux, C., Alloui, A., Diochot, S., Baron, A., Voilley, N., Blondeau, N., Escoubasp, Gélot, A., Cupo, A., Zimmer, A., Zimmer A.M., Eschalier, A., Lazdunski, M. (2007).** A tarantula peptide against pain via ASIC1 channels and opioid mechanisms. *Nat Neurosci*, 10(8):943-945.

Milescu, M., Bosmans, F., Lee, S., Alabi, A.A., Kim, J.I., Swartz, K.J. (2009).

Interactions between lipids and voltage sensor paddles detected with tarantula toxins. *Nat Struct Mol Biol*, 16(10):1080-1085.

Mourão, C.F.B., Oliveira, F.N.E., Carvalho, A.C., Arenas, C.J., Duque, H.M., Gonçalves, J.C., Macêdo, J.K., Galante, P., Schwartz, C.A., Mortari, M.R., Almeida, Santos, M.F., Schwartz, E.F. (2013). Venomic and pharmacological activity of *Acanthoscurria paulensis* (Theraphosidae) spider venom. *Toxicon*, 61:129–138.

Nazir, S.A., Dick, D.J., Coen, B.A., Sachs, F., Lab, M.J. (1996). Anti-arrhythmic action of *G. spatulata* venom in the ventricle of the

isolated Langendorff perfused guinea-pig heart. *J Physiol*, 494:111-112.

Suchyna, T.M., Johnson, J.H., Hamer, K., Leykam, J.F., Gage, D.A., Clemo, H.F., Baumgarten, C.M., Sachs, F. (2000). Identification of a peptide toxin from *Grammostola spatulata* spider venom that blocks cation-selective stretch-activated channels. *J Gen Physiol*, 115(5):583-598.

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<http://www.rompharm.ro/ro/isoflurane>