Non-clinical evidence supports anti-inflammatories as more effective medication than antihistamines against tarantula local effects envenomation

Bruno Ricardo Alves¹, Rafael Sutti¹, Pedro Ismael da Silva Jr.², Rogerio Bertani³, Jair Guilherme Santos-Junior¹, Thomaz Augusto Alves Rocha e Silva⁴, Alessandra Linardi¹

Affiliation:
¹Department of Physiological Sciences, Santa Casa de São Paulo School of Medical Sciences, São Paulo, SP, Brazil
²Laboratorio Especial de Toxinologia Aplicada, Instituto Butantan, São Paulo, SP, Brazil
³Laboratorio de Ecologia e Evolução, Instituto Butantan, São Paulo, SP, Brazil
⁴Faculdade Israelita de Ciências da Saúde Albert Einstein, São Paulo, SP, Brazil

Corresponding authors:
AL - Department of Physiological Sciences, Faculdade de Ciências Médicas da Santa Casa de São Paulo, Rua Dr. Cesário Motta Junior, 61, Vila Buarque, São Paulo, SP, 01221-020, Brazil.
Tel.: +(55)-11-33679038. e-mail: alinar@uol.com.br

TAARS – School of Nursing and Medicine Faculdade Israelita de Ciências da Saúde Albert Einstein, Avenida Professor Francisco Morato, 4293, Vila Sônia, São Paulo, SP, 05521-200, Brazil. Tel/FAX: +(55)-11-21511001. email: thomaz.silva@einstein.br
Abstract

Background: Tarantulas are the most common invertebrates pets, especially in North America and Europe. The most commercialized genera are from Southern Asia and tropical Americas, represented by Vitalius, found in Southeastern Brazil, and Brachypelma, common in Mexican desert. Bites by these spiders in humans occurs during manipulation and generally result in clinical manifestations such local pain, erythema and oedema, with the possibility of secondary local infection. Hence, the cases are usually treated with prescription free drugs such as antihistamines and anti-inflammatories.

Methods: In this work, we investigated the post treatment with commercial nonsteroidal and steroidal anti-inflammatories and anti-histamines administered by oral and intraperitoneal routes on rat paw oedema induced by venoms of V. dubius and B. smithi. Hydroplethysmometer standard oedema measurement and Evans blue extravasation were performed. Dose standardization experiments showed that the V. dubius is more potent than B. smithi, and doses were established at 30 µg/paw and 60 µg/paw respectively.

Results: The oral post-administration of ketoprofen (non-selective cyclooxygenase inhibitor) and prednisolone (steroidal anti-inflammatory) markedly reduced a paw oedema evoked by only V. dubius venom, but loratadine (H1-antihistamine) had negligible effect on rat paw oedema induced by both venoms. Intraperitoneal administration, ketoprofen (20 mg Kg⁻¹) and loratadine (5 mg Kg⁻¹) reduced the rat paw oedema induced by V. dubius and B. smithi while methylprednisolone (10 mg Kg⁻¹) only inhibited the oedema induced by V. dubius.
Conclusion: These results suggest that the post-treatment with nonsteroidal and steroidal anti-inflammatory drugs are more potent than antihistamines in attenuating the local effect induced by *V. dubius* and *B. smithi* venoms.

Keywords: paw oedema, *Vitalius dubius*, *Brachypelma smithi*, ketoprofen, loratadine, prednisolone, methylprednisolone.

Background

Spiders of the family Theraphosidae (suborder Mygalomorphae) are better known in many parts of the world as tarantulas and are worldwide commercialized as pets [1]. The tropical *Vitalius* and *Brachypelma* are one of the most common genus found with breeders in North America and Europe. *Vitalius dubius* occurs in the southern part of the Brazilian state of Minas Gerais and in the state of São Paulo [2]. *Brachypelma smithi*, one of the most popular tarantulas of the world also named mexican redknee tarantula, is found along the central Pacific coast of Mexico, from southern coastal Jalisco to north-western Oaxaca State and inland to the states of Mexico and Morelos [3].

The most common defensive behavior of species of such genera is the release of abdominal urticating hairs that elicit mild allergic reactions and seldom evolve to serious injuries [4]. Thereby, this friendly profile encourages breeders to manipulate the animals and bites occasionally occurs. Despite their large size and potentially large venom yields, bites by Theraphosidae spiders in humans generally result in only local clinical manifestations such local pain, erythema and oedema, but do not induce local necrosis or systemic effects [5, 6, 7, 8]. Besides the risk of infection, inflammation and swealling at the puncture site can arise. Based on the nine patients, Isbister [6] described that the main clinical effect of bites by Australian theraphosid spiders is severe local
pain, usually with puncture marks, but general systemic effects such as nausea and vomiting are uncommon. The puncture wounds from the spider's fangs require local wound care, monitoring for signs of infection, short-term analgesia and sometimes tetanus vaccine [9]. Such cases rarely lead to medical assistance, what makes it an underestimated casuistic. Nevertheless, spider collectors often assume to have enough knowledge to realize that symptomatic healing is enough for treatment and appeal to over-the-counter medication. More, many animals are obtained through illegal market, what leads breeders to avoid notifications of such accidents.

In this work, we propose to investigate the effect of oral and intraperitoneal post-treatment of clinical used nonsteroidal and steroidal anti-inflammatories and anti-histamines on the rat paw oedema and plasma extravasation induced by venoms of *Vitalius dubius* and *Brachypelma smithi*.

**Methods**

**Animals**

Male Wistar-Hanover rats (200-250 g) were obtained from Department of Physiological Sciences at Santa Casa de São Paulo School of Nursing and Medicine (Faculdade de Ciências Médicas da Santa Casa de São Paulo, FCMSCSP) and were housed 5/cage at 23°C on a 12 h light/dark cycle, with free access to food and water. The experimental protocols were approved by an institutional Committee for Ethics in Animal Experimentation (CEUA/FCMSCSP, protocol nº. 001/13) and the general ethical guidelines for animal use established by the Brazilian Society of Laboratory Animal Science (SBCAL, formerly the Brazilian College for Animal Experimentation - COBEA) and EU Directive 2010/63/EU for animal experiments were followed.
The *Vitalius dubius* and *Brachypelma smithi* spiders were acquired from field collection and the Instituto Butantan, respectively. This work was performed following the CITES recommendations and national licenses required (IBAMA SISBIO 58786-1 and CGen 010115/2014-5).

**Venom and drugs**

Venoms were obtained according to Rocha e Silva [10] for both species. Evans blue dye was purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Ketoprofen was purchased from Sanofi-Aventis Farmacêutica (Susano, SP, Brasil). Prednisolone was purchased from Mantecorp Indústria Química e Farmacêutica (Rio de Janeiro, SP, Brazil). Methylprednisolone was obtained from União Química Farmacêutica (São Paulo, SP, Brazil). Loratadine was purchased from Medley Indústria Farmacêutica (Campinas, SP, Brazil). Formamide was obtained from Dinâmica Química Contemporânea (Diadema, SP, Brazil) and isoflurane was from Cristália (Itapira, SP, Brazil).

**Paw oedema assay**

The rats were anaesthetised with isoflurane (inhaled). A subplantar injection of *V. dubius* venom (10 or 30 μg/paw) and *B. smithi* (30 or 60 μg/paw) were made into one paw on the rat. The paw volume was assessed immediately before venom, for the basal measurement and 30, 60, 120, 150 and 180 min after administration of venom, using a plethysmometer (model 7150, Ugo Basile). The final volume injected in the paw was always 0.1 ml. The same volume of saline was used in a control group. The increase in paw volume (ml) was calculated as the difference between the basal and final volumes [11]. N = 6 was used for all groups.

**Anti-inflammatory and antihistaminic activity**
After standardization of dosing, groups were divided in two experimental lines based on the route of application: intraperitoneal and oral routes. Four groups of rats were envenomed and received saline (control group, 1 mL Kg\(^{-1}\)), ketoprofen (20 mg Kg\(^{-1}\)), methylprednisolone (steroidal anti-inflammatory; 10 mg Kg\(^{-1}\)) or loratadine (5 mg Kg\(^{-1}\)) by intraperitoneal route. Another four groups were also envenomed and orally treated with saline (control group, 1 mL Kg\(^{-1}\)), ketoprofen (non-selective cyclooxygenase inhibitor; 50 mg Kg\(^{-1}\)), prednisolone (steroidal anti-inflammatory; 20 mg Kg\(^{-1}\)) or loratadine (H\(_1\)-antihistaminic drug; 10 mg Kg\(^{-1}\)). The equivalent corticosteroids prednisolone and methylprednisolone were used as the adequate preparation for the pharmacokinetics of the route of administration. In both experimental lines all drugs and saline were administered 15 min after respective venom inoculation.

**Vascular permeability assay**

The plasma extravasation measured by Evans blue assay was performed in a restricted group of animals because this assay implies in animal sacrifice and animal welfare committee recommended as maximum of thirty animals. Hence, the most prominent results obtained with paw oedema were transposed to this assay, following the same protocol of drugs administration. Evans blue (25 mg/kg, 2.5% w/v in 0.45% NaC1) was injected intravenously immediately before subplantar injection of *V. dubius* venom (30 µg/paw). Control groups received saline by the same route as the drugs. Two hours later, the animals were killed and dye exudate were measured [12, 13]. Briefly, animal paws were amputated at the tarsocrural joint and weighed on an analytical balance. Subsequently, each paw was chopped into small pieces and placed in a test tube with formamide 4 ml and then incubated in a water bath 57\(^{0}\)C for 24 h. The absorbance of the supernatants was measured at 619 nm and the concentration of Evans blue present in the
extracts was determined from a standard curve of the dye prepared in formamide. The amount of Evans blue dye was expressed as µg/g of paw and the results presented as the difference in the quantity of Evans blue between the oedematogenic paw and contralateral paw. N = 5 for venom control and 4 for each treatment.

**Statistical analysis**

Results are presented as means ± SD. The results were analyzed by the one way ANOVA for repeated measure considering as factor the period of oedema measured: 30, 60, 120, 150 and 180 min or one way ANOVA considering as factor the treatment. Fisher post-hoc was used when necessary. Values with p<0.05 were considered statistically significant.

**Results**

**Paw oedema induced by V. dubius and B. smithi venoms**

Intraplantar injection of *V. dubius* venom (10 and 30 µg/paw) in the rat hind-paw caused a dose and time-dependent oedema (Fig. 1), when compared to Saline groups. The one way ANOVA for repeated measures shows that there were differences in the group [F(2,13)=71.66], in the time factors [F(5,65)=108.99], as well as in the group vs. time interaction [F(10,65)=20.15]. For further studies, *V. dubius* venom was administered at the dose of 30 µg/paw. We also observed a dose and time-dependent oedema induced by *B. smithi* venom (30 and 60 µg/paw; Fig 1), when compared to Saline group. The ANOVA for repeated measures detected significant differences in the group [F(2,14)=69.77], the time factors [F(5,70)=103.93] and in the group vs. time interaction [F(10,70)=25.84]. *B. smithi* venom was administered at the dose of 60 µg/paw for subsequent studies.
Figure 1 – Dose dependent hind paw oedema evoked by *V. dubius* and *B. smithi* venoms. Note the higher potency of the *Vitalius* venom. *p<0.05 compared to saline; #p<0.05 compared to other dose.
Table 1 summarizes the statistical outcomes of post-treatment experiments, presented in the following items.

Table 1 – Summary table of statistics outcomes comparing different venoms, treatments, routes and times for the hind paw oedema. Columns shows the P values of the comparison of underlined treatment with the other two, respectively separated by bars. The significances of P values are in bold and indicated in figures 2 and 3.

|                      | Vitalius dubius      | Brachypelma smithi | P values* | F value**            |
|----------------------|----------------------|--------------------|-----------|----------------------|
|                      | NSAID x control /    | Corticosteroid x control / | Antihistamine x control / | NSAID x control / | Corticosteroid x control / | Antihistamine x NSAID |
|                      | x corticosteroid     | x antihistamine    | x NSAID   | x corticosteroid     | x antihistamine    |                     |
| Intrapertoneal       |                      |                   |           |                      |                     |                     |
| 30                   | 0.541 / 0.104        | 0.026 / 4x10^-4    | 0.036 / 0.007 | 0.243 / 0.009        | 0.117 / 2x10^-4     | 0.0002 / 0.005      |
| 60                   | 0.118 / 0.003        | 0.0001 / 0.002     | 0.460 / 0.404 | 0.008 / 0.005        | 0.502 / 2x10^-4     | 3x10^-4 / 0.050     |
| 90                   | 0.042 / 0.404        | 0.005 / 0.111      | 0.201 / 0.441 | 10^-7 / 4x10^-4      | 0.212 / 10^-5       | 4x10^-7 / 0.674     |
| 120                  | 0.002 / 0.847        | 0.001 / 0.212      | 0.039 / 0.290 | 7x10^-8 / 4x10^-5    | 0.800 / 0.001       | 0.002 / 0.212       |
| 180                  | 0.004 / 0.995        | 0.005 / 0.484      | 0.029 / 0.480 | 3x10^-7 / 2x10^-4    | 0.557 / 6x10^-8     | 10^-9 / 0.614       |
|                      |                      |                   |           |                      |                     |                     |
| Oral                 |                      |                   |           |                      |                     |                     |
| 30                   | 0.691 / 0.803        | 0.881 / 0.206      | 0.158 / 0.309 | 0.310 / 0.150        | 0.666 / 0.072       | 0.168 / 0.711       |
| 60                   | 0.333 / 0.567        | 0.690 / 0.568      | 0.333 / 1.000 | 0.579 / 0.325        | 0.666 / 0.167       | 0.340 / 0.688       |
| 90                   | 0.173 / 0.503        | 0.487 / 0.321      | 0.093 / 0.747 | 0.734 / 0.538        | 0.341 / 0.220       | 0.781 / 0.558       |
| 120                  | 0.002 / 0.225        | 0.049 / 0.518      | 0.009 / 0.568 | 0.711 / 0.479        | 0.734 / 0.208       | 0.356 / 0.579       |
| 150                  | 0.032 / 0.881        | 0.022 / 0.456      | 0.119 / 0.551 | 0.829 / 0.256        | 0.356 / 0.126       | 0.538 / 0.689       |
| 180                  | 0.054 / 0.673        | 0.020 / 0.456      | 0.108 / 0.746  | 0.758 / 0.622        | 0.424 / 0.142       | 0.498 / 0.326       |
|                      |                      |                   |           |                      |                     |                     |

*Fisher post hoc test; ** one way ANOVA of repeated measures

**Effect of intraperitoneal administration (i.p.) of ketoprofen, loratadine and prednisolone on paw oedema**

Figure 2 illustrates the effect of intraperitoneal post-treatment (15 min after venoms injection) of ketoprofen (20 mg Kg^-1), loratadine (5 mg Kg^-1) and
Figure 2 – Hind paw oedema evoked by *V. dubius* (A, 30 μg) and *B. smithi* (B, 60 μg) venoms in animals treated 15 minutes after envenomation with intraperitoneal saline (control group, 1 mL Kg⁻¹), ketoprofen (10 mg/kg), methylprednisolone (20 mg/kg) or loratadine (5 mg/kg). Legends are valid for both A and B graphics. Number indicates p<0.05 of respective comparison.
methylprednisolone (10 mg Kg⁻¹) on paw oedema induced by V. dubius venom (Fig. 3A) and B. smithi venom (Fig. 3B). For V. dubius venom, the repeated measures ANOVA shows that there were differences in the group and time factors and the group vs. time interaction (Table 1). Regarding B. smithi venom, same statistical analysis also describes differences in the group and time factors but in the group vs. time interaction there was no significant difference (Table 1). The Fisher post-hoc test shows that the intraperitoneal pos-administration of methylprednisolone significantly reduced the paw oedema induced by V. dubius venom at all subsequent intervals from 30 min, when compared to Control group. In addition, ketoprofen and loratadine significantly reduced the paw oedema in 90 and 120 min towards the end of experiment, respectively. Otherwise, significantly reduction in oedema evoked by B. smithi venom was not observed with methylprednisolone, but loratadine from 30 and ketoprofen from 60 until 180 min.

**Effect of oral administration (p.o.) of ketoprofen, loratadine and prednisolone on paw oedema**

Figure 3 shows the effect of oral pos-treatment (15 min after venoms into the paw) of ketoprofen (50 mg Kg⁻¹), loratadine (10 mg Kg⁻¹) and prednisolone (20 mg Kg⁻¹) on paw oedema induced by V. dubius venom (Fig. 4A) and B. smithi venom (Fig. 4B). For V. dubius venom, the repeated measures ANOVA shows that there were differences in the group and time factors, as well as in the group vs. time interaction. However, for B. smithi venom, there were differences only in the group and time factors, but there were no significant differences in the group vs. time interaction (Table 1). This indicates that oral prednisolone and ketoprofen are able to diminish the oedema caused by B. smithi venom as a hole treatment and an increase in the number of animals may reach significance in group vs. time interaction. The Fisher post-hoc test shows that
Figure 3 - Hind paw oedema evoked by *V. dubius* (A, 30 μg) and *B. smithi* (B, 60 μg) venoms in animals treated 15 minutes after envenomation with oral saline (control group, 1 mL Kg\(^{-1}\)), ketoprofen (50 mg/kg), prednisolone (20 mg/kg) or loratadine (10 mg/kg). Legends are valid for both A and B graphics. Number indicates p<0.05 of respective comparison.
the oral post-treatment with ketoprofen significantly reduced the paw oedema induced by *V. dubius* venom at intervals of 120 and 150, when compared to Control group. However, the prednisolone post-treatment significantly reduced the paw oedema only at intervals from 120 min towards the end of experiment. Loratadine significantly alter the paw oedema induced by *V. dubius* venom only at 120 min.

**Effect of intraperitoneal (i.p.) or oral (p.o.) administration of ketoprofen, loratadine and prednisolone or methylprednisolone on vascular exudation induced by *V. dubius* venom**

The time interval of 120 min was chosen to evaluate the Evans blue extravasation induced by *V. dubius* venom in rats. The one way ANOVA detected significant differences in the groups [F(2.14)=10.8] after intraperitoneal post-treatment, as well as it was observed differences between groups [F(2.11)=12.10] after oral post-treatment (Fig. 4). The Fisher pos-hoc test showed that the intraperitoneal treatment of ketoprofen (20 mg Kg⁻¹) and methylprednisolone (10 mg Kg⁻¹) significantly reduced the Evans blue extravasation induced by *V. dubius* venom, when compared to Control group. Regarding the oral post-treatment, Fisher pos-hoc test detected that the ketoprofen (50 mg Kg⁻¹) and prednisolone (20 mg Kg⁻¹), but not loratadine (5 mg Kg⁻¹), significantly inhibited the Evans blue extravasation, when compared to Control group.

**Discussion**

American tarantula spiders are usually not aggressive and easily manipulable often living in regions with easy access for being captured. Despite the concern of conservationists about the increase of illegal commerce, these spiders have been kept as pets mainly in North America and Europe, but also in many other parts of the world.
Figure 4 – Effect of oral (p.o., black bars) or intraperitoneal (i.p., white bars) administration of saline (venom group), ketoprofen (20 mg/kg i.p and 50mg/kg; p.o), methylprednisolone (10 mg/kg; i.p.)/prednisolone (20 mg/kg; p.o.) and loratadine (5 mg/kg; i.p.) on Evans blue extravasation induced by V. dubius venom after 120 minutes of venom injection. Each column represent mean ± standard error (N = 5 for venom control and 4 for each treatment). *p<0.05 vs. control (venom alone)
Nevertheless, stressed or hungry animals may display defensive behavior such as the release of urticating hairs or even biting an unsuspected owner.

The bite of the tarantulas is quite painful mainly due to the size of the fangs, but few cases of envenomation are reported, probably due to low toxicity to humans [5, 8] and therefore it is underreported to health systems. Hence, mild clinical consequences such as swelling and moderate pain rarely requires medical assistance, mainly amongst experienced breeders that usually appeal to over the counter medicines.

Although reports of minor effects in humans, a number of studies have demonstrated significant toxicity of theraphosid venoms in various animals, including rats, mice, cats, birds and dogs [14, 15, 16]. In addition, it is also recognized that theraphosid spiders can cause more severe effects in domestic animals, including death [6, 17, 18].

The venom composition of the two species considered in this work has been studied and share characteristics such as the presence of hyaluronidase and lack of phospholipase and proteinolytic activity [10, 19]. *V. dubius* is the only specie of the genus that has already published data on venom composition, including an ionotropic blocker polyamine with yet antimicrobial activity [20, 21]. The described oedema activity [22] was not associated to a specific toxin since it was demonstrated to be induced by various pathways. Otherwise, the venoms of the *Brachypelma* genus have been widely studied, comprising insecticidal and neurotoxic activity [23, 24], with small molecules related to tissue toxicity [25]. Despite scarce macromolecules, theraphosid spiders produce a wide range of peptides that can differ between species of the same genera and usually have selective ligand properties [26] that may also elicit inflammation and local reactions.
Our results demonstrated that the venom of *V. dubius* and *B. smithi* induced a paw oedema in rats, with difference in the respective doses, what evidences a higher potency of the South American spider. The local effect of the venom of the *V. dubius* was first inhibited by the corticosteroid, followed by ketoprofen and loratadine, respectively, when drugs were administered intraperitoneally. Concerning the oral route, the efficacy of loratadine was statistically lower than other drugs when compared to control group. These observations meet previous results concerning the pharmacology of the oedema caused by *V. dubius* that showed no participation of histamine and bradykinin, but a significant action of serotonin, eicosanoids, neurokinins and nitric oxide [22].

On the other hand, the local action of the *B. smithi* venom have suffered intense inhibition by loratadine and ketoprofen but not by methylprednisolone when administered intraperitoneally. The oral route for the same drugs, although observed only as a group effect, conversely demonstrated oedema inhibition by corticosteroid, followed by ketoprofen and lack of loratadine effect, opening the room for the discussion about pharmacological differences of the venoms. The results indicate a possible role of histamine in the oedema caused by *B. smithi* venom, although this component was not found in the composition of previously studied venom [25]. Comparable paw oedema was observed with *B. epicureanum* but no pathways or toxins were investigated [27]. These results are evidences of the presence of peptides that may elicit inflammation in this venom.

The plasma extravasation measured by Evans blue assay was performed only with *Vitalius* venom because of the higher potency compared to *Brachypelma*. Loratadine was tested orally to check if its efficacy was indeed lower, since the intraperitoneal administration demonstrated oedema healing. Surprisingly, ketoprofen
was the most efficient drug on avoiding plasma extravasation, with significative reduction through both routes of administration, while corticosteroid was significant only through intraperitoneally administration. Finally, oral loratadine or corticosteroid were not different from control, evidencing a primary role of cyclooxygenase derivates in plasma extravasation evoked by V. dubius venom.

There are few studies related to the mechanisms involved in local manifestations in accidents caused by theraphosid spiders. It was demonstrated that tarantulas may produce pro-inflammatory and nociceptive peptides acting as capsaicin receptor agonist and pain-related sodium channel activators, respectively [28, 29]. Our group demonstrated the participation of neurokinins, cyclooxygenase and nitric oxide pathways on skin oedema caused by V. dubius venom [22]. It is remarkable that most of the studies that investigate the mechanisms of action of venom and/or its fractions, administered drugs before the envenomation, i.e. before the onset of the inflammatory response by venom [30, 31, 32]. Otherwise, the importance of post-treatment studies of envenomation was recently corroborated. The systemic effects of various Poecilotheria genera venoms has been studied in mice and demonstrated several neurological and muscular symptoms. The authors demonstrated that post-treatments with at least three drugs were able to avoid cramps and movement stereotytpy [33].

Also, a comparison between intraperitoneal and oral routes arises. It was observed that intraperitoneal administration of drugs was more effective when compared the oral administration, especially when considering the paw oedema induced by B. smithi venom. Oral administration is considered to be safe, efficient and easily accessible with minimal discomfort to the patient compared to other routes of administration. However, the absorption of the drug may be reduced due to digestive enzymes, gastrointestinal tract motility, pH, and especially by the hepatic first-pass
metabolism. It is known that antihistamines are extensively transformed in liver to inactive metabolites or a minor fraction of active ones such as desloratadine [34]. Otherwise, the pharmacokinetic pathways of (methyl)prednisolone are complex and protein bound distribution and interconversion between prednisolone and prednisone [35] may explain the lack of inhibition of *B. smithi* venom when administered intraperitoneally.

The use of NSAIDs such as ketoprofen are often related to pain control, but our work demonstrated the benefits of these drugs in avoiding the local effect, as a second hand effect. Also, systemic (i.e. oral) corticosteroids showed a significative action against envenomation. However, concerning the side effects of NSAIDs and corticosteroids, the first is usually related to stomach discomfort in a short term use [36], while the second shall be avoided through systemic routes because of potential effects on immune system [37], since local infection is a concern during tarantula envenomation. The action of loratadine may be useful in cases which is the only available drug or with the arousal of a systemic allergic reaction, but the liver first pass metabolism may not allow a locally effective concentration.

**Conclusion**

The main contribution of the present work is the comparison of three commercially available drugs to treat local oedema caused by tarantulas envenomation. Our results showed that anti-inflammatory medicines are more effective in healing local effects than antihistamines. If the systemic (oral) strategy is chosen, NSAIDs may be a better choice, corticosteroids shall be used topically and antihistamines reserved for the case of allergic reactions.
Abbreviations

ANOVA – analysis of variance
B. smithi – Brachypelma smithi
CEUA - Committee for Ethics in Animal Experimentation
CGen – Commission for Biodiversity Genetic Access
COBEA - Brazilian College for Animal Experimentation
COX – cyclooxygenase
FCMSCSP – Santa Casa de São Paulo School of Medicine
H1 – histamine receptor 1
i.p. – intraperitoneal
IBAMA – Brazilian Institute for Environment
NSAIDs – non steroidal anti-inflammatory drugs
p.o. – oral administration
SBCAL - Brazilian Society of Laboratory Animal Science
SD – standard deviation
SISBIO – Biological Sampling License System
V. dubius – Vitalius dubius

Acknowledgements

The authors would like to thank Fundação Florestal de São Paulo, the managers of PETAR, Intervales and Serra do Mar-Curucutu State Parks and Centro de Controle de Zoonoses de Itu.

Availability of data and materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests

Authorship Contributions

Participated in research design: AL, JGSJr, TAARS and BRA
Conducted experiments: BRA, RS and AL
Contributed new reagents or analytic tools: RB, PISJr, TAARS and RS
Performed data analysis: JGSJr, AL and TAARS
Wrote or contributed to the writing of the manuscript: AL, TAARS, JGSJr, PISJr and RB

Ethics approval
The experimental protocols were approved by an institutional Committee for Ethics in Animal Experimentation (CEUA/FCMSCSP, protocol n°. 001/13) and the general ethical guidelines for animal use established by the Brazilian Society of Laboratory Animal Science (SBCAL, formerly the Brazilian College for Animal Experimentation - COBEA) and EU Directive 2010/63/EU for animal experiments were followed.
References

1. Actman J. [Internet]. The illegal market for tarantulas is hairy business. National Geographic [cited 2018 October 13]. Available from: https://www.nationalgeographic.com/animals/2018/10/tarantula-illegal-wildlife-trade/?fbclid=IwAR2qd-VijiBhB1ttTt8TrpkA4AFuSxN3KT0NT36ya%E2%80%A6.

2. Bertani R. Revision, cladistic analysis, and zoogeography of Vitalius, Nhandu and Proshapalopus, with notes on other Theraphosinae genera (Araneae, Theraphosidae). Arq Zool. 2001 Apr 20; 36(3): 265-356.

3. West RC. The Brachypelma of Mexico. Journal of the British Tarantula Society. 2005 Aug 01; 20(4): 108-119.

4. Shrum K, Robertson D, Baratz KH, Casperson TJ, Rostvold JA. Keratitis and retinitis secondary to tarantula hair. Arch Ophthalmol. 1999 Aug;117(8):1096-7. doi: 10.1001/archophthalmol.117.8.1096

5. Lucas SM, Da Silva Júnior PI, Bertani R, Cardoso JL. Mygalomorph spider bites: a report on 91 cases in the state of São Paulo, Brazil. Toxicon. 1994 Oct; 32: 1211-15. doi: 10.1016/0041-0101(94)90350-6

6. Isbister GK, Seymour J., Gray MR, Raven RJ. Bites by spiders of the family Theraphosidae in humans and canines. Toxicon. 2003 Mar; 41: 519-24. doi: 10.1016/S0041-0101(02)00395-1

7. Saucier JR. Arachnid envenomation. Emerg med Clin N Am. 2004 May; 22(2): 405-22. doi: 10.1016/j.emc.2004.01.006

8. Ahmed N, Pinkham M, Warrell DA. Symptom in search of a toxin: muscle spasms following bites by Old World tarantula spiders (Lampropelma nigerrimum, Pterinochilus murinus, Poecilotheria regalis) with review. Q J Med. 2009 Sep 23; 102(12): 851-7. doi:10.1093/qjmed/hcp128
9. Kelley T, Wasserman G. The dangers of pet tarantulas: experience of the Marseilles Poison Centre. J Toxicol Clin Toxicol. 1998 Jul 29; 36(1-2):51-3. doi: 10.3109/15563659809162586

10. Rocha-e-Silva TA, Sutti R, Hyslop S. Milking and partial characterization of venom from the Brazilian spider *Vitalius dubius* (Theraphosidae). Toxicon. 2009 Jan; 53(1):153-61. doi: 10.1016/j.toxicon.2008.10.026

11. Ferreira SH. A new method for variations of rat paw volume. J Pharm Pharmacol. 1979 Sep;31(9):648. doi: 10.1111/j.2042-7158.1979.tb13616.x

12. Udaka K, Takeuchi Y and Movat HZ. Simple method for quantitation of enhanced vascular permeability. Proc Soc Exp Biol Med. 1970 Apr; 133(4):1384-7. doi: 10.3181/00379727-133-34695

13. Medeiros MV, Binhara IM, Moreno Júnior H, Zatz R, De Nucci G, Antunes E. Effect of chronic nitric oxide synthesis inhibition on the inflammatory responses induced by carrageenin in rats. Eur J Pharmacol. 1995 Oct 16; 285(2):109-14. doi: 10.1016/0014-2999(95)00332-f

14. Bucherl W. Spiders. In: Bucherl W, Buckley EE, editors. Venomous Animals and their Venoms. London: Academic Press; 1971. p. 197-277.

15. Bettini S and Brignoli PM. Review of the spider families, with notes on the lesser-known poisonous forms. In: Bettini S, editor. Arthropod Venoms. Berlin: Springer-Verlag; 1978. p. 103-11.

16. Atkinson RK. A comparison of the toxicity of the venoms of twelve common Australian spider species on rodent vital organ systems. Comp. Biochem. Physiol. 1993 Nov; 106(3): 639-42. doi: 10.1016/0742-8413(93)90220-f

17. Robinson G, Griffin G. Effects of a bite from a barking spider (*Selenocosmia stirlingi* Hogg). Victorian Naturalist. 1985; 100:116-7.
18. Raven RJ. Spiders (other arachnids and myriapods). In: Ryan M, Burwell C, editors. Wildlife of Tropical North Queensland. Brisbane: Queensland Museum, 2000. p. 21-41.

19. Rodríguez-Rios L, Díaz-Peña LF, Lazcano-Pérez F, Arreguín-Espinosa R, Rojas-Molina A, García-Arredondo A. Hyaluronidase-like enzymes are a frequent component of venoms from theraphosid spiders. Toxicon. 2017 Sep 15; 136:34-43. doi: 10.1016/j.toxicon.2017.07.001

20. Rocha-E-Silva TA, Rostelato-Ferreira S, Leite GB, da Silva PI Jr, Hyslop S, Rodrigues-Simioni L. VdTX-1, a reversible nicotinic receptor antagonist isolated from venom of the spider Vitalius dubius (Theraphosidae). Toxicon. 2013 May 10; 70:135‐141. doi:10.1016/j.toxicon.2013.04.020

21. Sutti R, Rosa BB, Wunderlich B, da Silva Junior PI, Rocha E Silva TAAD. Antimicrobial activity of the toxin VdTX-I from the spider Vitalius dubius (Araneae, Theraphosidae). Biochem Biophys Rep. 2015 Sep 28; 4:324-328. doi:10.1016/j.bbrep.2015.09.018

22. Rocha-e-Silva TA, Linardi A, Antunes E, Hyslop S. Pharmacological Characterization of the Edema Caused by Vitalius dubius (Theraphosidae, Mygalomorphae) Spider Venom in Rats. J Pharmacol Exp Ther. 2015 Oct 15; 356(1): 13-9. doi: 10.1124/jpet.115.226787

23. Corzo G, Bernard C, Clement H, et al. Insecticidal peptides from the theraposid spider Brachypelma albiceps: an NMR-based model of Ba2. Biochimica et Biophysica Acta. 2009 Apr 14;1794(8):1190-1196. DOI: 10.1016/j.bbabap.2009.04.004.

24. Zhong Y, Song B, Mo G, et al. A novel neurotoxin from venom of the spider, Brachypelma albopilosum. Plos one. 2014 Oct 19; 9(10):e110221. DOI: 10.1371/journal.pone.0110221.
25. Clement H, Alagón A, Possani L, Odell GV. Venom Components of *Brachypelma vagans*, a Mexican Tarantula. J Venom Anim Toxins. 2001 Dec 1; 7(2): 337. doi: 10.1590/S0104-79302001000200033

26. Escoubas P, Célérier ML, Nakajima T. High-performance liquid chromatography matrix-assisted laser desorption/ionization time-of-flight mass spectrometry peptide fingerprinting of tarantula venoms in the genus Brachypelma: chemotaxonomic and biochemical applications. Rapid Communications in Mass Spectrometry: RCM. 1996 Dec 31; 11(17):1891-1899. DOI: 10.1002/(sici)1097-0231(19971111:17<1891::aid-rcm94>3.0.co;2-x.

27. García-Arredondo A, Rodríguez-Rios L, Díaz-Peña LF, Vega-Ángeles R. Pharmacological characterization of venoms from three theraphosid spiders: *Poecilotheria regalis, Ceratogyrus darlingi* and *Brachypelma epicureanum*. J.Venom. An. Tox. incl. Trop. Dis. 2015 Aug 11; 21: 1-9. doi:10.1186/s40409-015-0017-8

28. Siemens J, Zhou S, Piskorowski R, Nikai T, Lumpkin EA, Basbaum AI, King D, Julius D. Spider toxins activate the capsaicin receptor to produce inflammatory pain. Nature. 2006 Nov 09; 444: 208-12. doi: 10.1038/nature05285

29. Osteen JD, Herzig V, Gilchrist J, Emrick JJ, Zhang C, Wang X, Castro J, Garcia-Caraballo S, Grundy L, Rychkov GY, Weyer AD, Dekan Z, Undheim EA, Alewood P, Stucky CL, Brierley SM, Basbaum AI, Bosmans F, King GF, Julius D. Selective spider toxins reveal a role for the Nav1.1 channel in mechanical pain. Nature. 2016 Jun 05; 534(7608): 494-499. doi: 10.1038/nature17976

30. Zanchet EM, Cury Y. Peripheral tackykinin and excitatory amino acid receptors mediate hyperalgesia induced by *Phoneutria nigriventer* venom. Eur J Pharmacol. 2003 Apr 25; 467(1-3):111-8. doi: 10.1016/s0014-2999(03)01604-2.
31. Costa SK, Starr A, Hyslop S, Gilmore D, Brain SD. How important are NK1 receptors for influencing microvascular inflammation and itch in the skin? Studies using Phoneutria nigriventer venom. Vascul Pharmacol. 2006 Oct 16; 45: 209-14. doi: 10.1016/j.vph.2005.08.025

32. Paludo KS, Biscaia SM, Chaim OM, Otuki MF, Naliwaiko K, Dombrowski PA, Franco CR, Veiga SS. Inflammatory events induced by brown spider venom and its recombinant dermonecrotic toxin: a pharmacological investigation. Comp Biochem Physiol C Toxicol Pharmacol. 2009 Apr; 149: 323-33. doi: 10.1016/j.cbpc.2008.08.009

33. Andreev-Andrievskiy A, Popova A, Lagereva E, Osipov D, Berkut A, Grishin E, Vassilevski A. Pharmacological analysis of Poecilotheria spider venoms in mice provides clues for human treatment. Toxicon. 2017 Aug 12; 138: 59-67. doi: 10.1016/j.toxicon.2017.08.013

34. Aratyn-Schaus Y, Ramanathan R. Advances in high-resolution MS and hepatocyte models solve a long-standing metabolism challenge: the loratadine story. Bioanalysis 2016 Jul 26; 8(16): 1645-62. doi: 10.4155/bio-2016-0094.

35. Bergmann TK, Barraclough KA, Lee KJ, Staatz CE. Clinical pharmacokinetics and pharmacodynamics of prednisolone and prednisone in solid organ transplantation. Clin Pharmacokinet. 2012 Sep 7; 51: 711-41. doi: 10.1007/s40262-012-0007-8.

36. Chawla G, Ranjan C, Kumar J, Siddiqui AA. Chemical Modifications of Ketoprofen (NSAID) in Search of Better Lead Compounds: A Review of Literature From 2004-2016. Antiinflamm Antiallergy Agents Med Chem. 2017 Apr 16; 15(3): 154-77. doi: 10.2174/1871523016666170217094722.

37. Kobayashi-Sakamoto M, Tamai R, Isogai E, Kiyoura Y Gastrointestinal colonisation and systemic spread of Candida albicans in mice treated with antibiotics
and prednisolone. Microb Pathog. 2018 Apr; 117:191-199. doi: 10.1016/j.micpath.2018.02.043.