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ORIGINAL ARTICLE

Pharmacological Characterization of Mouse Hind Paw Edema Induced by *Parachartergus fraternus* Venom

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Abstract— Stings from the wasp *Parachartergus fraternus* occur throughout Latin America, and edema followed by pain is the main symptom presented by victims. This often limited inflammatory event has not been characterized for this species. In this work, we identified the mechanisms and possible mediators involved in this response. *P. fraternus* venom (100, 200, and 400 μg/kg) was injected into the hind paws of mice, and edema was evaluated at intervals of 10 min for up to 60 min and at 120, 240, and 1440 min using a digital plethysmometer. The peak of edema was observed at 10 min with a dose of 200 μg/kg. A reduction in edema was observed with indomethacin (58.1%), celecoxib (44.5%), MK886 (30.8%), and dexamethasone (53.2%). Loratadine, cimetidine, and cyproheptadine treatment reduced the edema by 54.2%, 63.9%, and 84.4%, respectively, compared with the control. Captopril and L-NAME inhibited 42.5% and 69.8%, respectively, of the edema. These results showed that the edema induced in mice by *P. fraternus* venom occurs early and is mediated by arachidonic acid derivatives, vasoactive amines, and nitric oxide. Together, these mediators amplify the inflammatory process, with emphasis on histamine and serotonin in triggering the edematogenic response, being more effective the use of cyproheptadine in the therapeutic approach.

KEY WORDS: wasp venom; receptor antagonists; inflammatory mediators.

INTRODUCTION

Wasps are considered venomous animals that belong to the order Hymenoptera, which also includes bees and ants. These insects may be solitary or social [1], and due to adaptations to living with humans, social wasps often build their nests in urban areas, attacking in situations where they feel threatened [2].

The sting of these wasps causes reactions such as increased vascular permeability [3], edema, prolonged pain, local erythema, urticaria, ulceration, headache, dizziness, nausea, loss of reflexes, and allergic reactions, which may induce anaphylactic shock, rhabdomyolysis, and renal, hepatic, and pancreatic injury [4]. A study conducted in the USA noted that between 2008 and 2015, bees and

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wasps accounted for most of the deaths caused by venomous animals [5], indicating that these accidents occur frequently and can be fatal.

Among the species of wasps found in Latin America is *Parachartergus fraternus*, which belongs to the subfamily Polistinae [6] and is considered social wasps. This insect has the ability to irreversibly paralyze its prey, a behavior described only for solitary wasps [7]; additionally, this insect will spray venom into an observer's eyes when it feels threatened, inducing allergic reactions [8].

The venom of wasps is composed of biogenic amines, proteins, and peptides [9], and these compounds may synergistically or antagonistically interact to produce different reactions in victims. One of the main symptoms presented by the victims of a *P. fraternus* sting is swelling, followed by pain and inflammation [10, 11]. Swelling is a local reaction; however, depending on the affected region, such as the oropharynx, swelling can result in compromised and closed airways, and most deaths are due to severe respiratory symptoms [10]. The paw edema induced by *P. fraternus* venom and the pathways and mediators involved have not yet been pharmacologically characterized. Thus, the objective of this work was to identify the mechanisms and possible mediators involved in this response.

MATERIALS AND METHODS

Animal

Male Swiss mice (18–25 g) were obtained from the Federal University of Mato Grosso do Sul (UFMS), Brazil, and maintained under standard laboratory conditions. The animals were housed at 22 ± 2 °C on a 12-h light/dark cycle with free access to food and water. Seven animals were included in each group. All experiments were approved by the Ethics Committee on Animal Experimentation of the UFMS (protocol 764/2016) and were conducted in accordance with the National Institutes of Health Regulations on the Use and Care of Animals for Scientific Purposes.

Venom

P. fraternus wasps were collected in Distrito Federal, Brazil, and the collection of wasp specimens was authorized by the Chico Mendes Institute for Biodiversity Conservation of Brazil (license number 21723-1). The specimens were frozen and then stored at − 20 °C for 24 h. After this period, venom sacs were dissected from the wasps, macerated in a 1:1 (v:v) acetonitrile/water solution and centrifuged at 5000 rpm for 10 min at room temperature. To obtain the crude venom, the supernatant was collected, frozen, and dried under vacuum. The venom was stored at − 20 °C and diluted at the time of use.

Evaluation of Paw Edema

Paw edema was evaluated according to the method described by Winter et al. [12]. Paw edema was induced in the hind paw of the mice by intraplantar injection (i.pl.) of *P. fraternus* venom (Pfv) at doses of 100, 200, and 400 μg/kg in a volume of 40 μL/paw. The contralateral paw was used as a control, which was injected with the same volume of vehicle (saline). A group in which vehicle was injected into both paws was also established. The course of the edema was monitored by measuring the thickness of footpad swelling at 10-min intervals up to 60 min and after 120, 240, and 1440 min using a digital plethysmometer (Insight®). Paw edema was determined by the difference between the volumes of the control and the stimulated paw. The results are expressed in milliliters.

Pharmacological Modulations

To evaluate the pathways involved in paw edema, drugs were administered before i.pl. injection of the venom. Indomethacin (non-selective cyclooxygenase (COX) inhibitor, 15 mg/kg), celecoxib (COX-2 inhibitor, 10 mg/kg), dexamethasone (phospholipase A_2/PLA_2 inhibitor, 3 mg/kg), MK886 (leukotriene/LT synthesis inhibitor, 3 mg/kg), loratadine (histamine H_1 receptor antagonist, 5 mg/kg), cimetidine (histamine H_2 receptor antagonist, 1 mg/kg), or cyproheptadine (dual antagonist of histamine H_1 and serotonin 5-HT receptors, 3 mg/kg) was administered *per os* (p.o.) 60 min before the injection of the venom. Captopril (inhibitor of angiotensin-converting enzyme (ACE), 10 mg/kg) and N(ω)-nitro-L-arginine methyl ester (L-NAME; nonselective inhibitor of nitric oxide synthesis, 50 mg/kg) were intraperitoneally injected (i.p.) 60 min and 30 min before the injection of the venom, respectively. Drugs were purchased from Sigma-Aldrich®, and the doses were based on the literature [13–20]. Edema was evaluated as previously described at 10-min intervals up to 60 min and after 120 and 240 min.

Statistical Analysis

The results are expressed as the mean ± standard error of the mean. Differences between groups were analyzed by one- or two-way ANOVA followed by the Bonferroni test;

p values less than 0.05 were considered significant. The area under the curve (AUC) and all analyses were performed using GraphPad Prism 5 software (GraphPad Software Inc., USA).

RESULTS

Paw Edema Induced by Pfv

The doses of 100, 200, and 400 μg/kg Pfv induced the formation of edema in the paws of mice. The peak of edema occurred with a dose of 200 μg/kg between 10 and 30 min, persisting up to 240 min. Only the dose of 400 μg/kg induced prolonged edema, which was observed up to 24 h after the injection of the venom (Fig. 1).

An increased edematogenic response was observed in animals treated with the 200-μg/kg dose between 10 min (0.147 ± 0.011 mL) and 30 min (0.137 ± 0.010 mL) compared with animals that received saline on both paws (10 and 30 min, 0.018 ± 0.007 mL and 0.024 ± 0.005 mL, respectively).

After this period, paw edema regressed up to 50 min, with a subsequent increase in the paw volume of the animals, which persisted up to 240 min. The peak of the edema was at 10 min, and at that time point, the highest increase in paw volume among the doses was the 200-μg/kg dose (100 μg/kg, 0.122 ± 0.010 mL, and 400 μg/kg, 0.102 ± 0.010 mL). Thus, the dose of 200 μg/kg over the period from 10 to 240 min was selected to evaluate the pathways and mediators involved in this effect.

Pathways and Mediators Involved in the Paw Edema Induced by Pfv

Involvement of the Arachidonic Acid Pathway

Indomethacin inhibited edema in all the analyzed time points, with 58.1% reduction at 10 min (peak of the inflammatory response). At the same time point, celecoxib and dexamethasone also exerted their maximum effects, with reductions of 44.5% and 53.2%, respectively. MK886, however, reduced edema by 30.8% (Fig. 2).

Analysis of the AUC of Pfv indicated an $AUC_{0–240\ min}$ of 24.37 ± 0.5 mL min. Indomethacin was more effective ($AUC_{0–240\ min}$ of 6.17 ± 0.8 mL min) at reducing paw edema than dexamethasone ($AUC_{0–240\ min}$ of 11.09 ± 0.6 mL min), celecoxib ($AUC_{0–240\ min}$ of 16.3 ± 0.7 mL min), and MK886 ($AUC_{0–240\ min}$ of 12.76 ± 1.0 mL min) (Fig. 2e).

Involvement of Vasoactive Amines and Nitric Oxide

Loratadine, cimetidine, captopril, and L-NAME reduced edema by 54.2%, 63.9%, 42.5%, and 69.8%, respectively, at 10 min. On the other hand, cyproheptadine showed a greater efficacy with an 84.4% reduction in the maximum observed inflammatory response.

According to Fig. 3a, b, which considers the histamine receptors H_1 and H_2, respectively, loratadine presented an $AUC_{0–240\ min}$ of 7.73 ± 0.4 mL min, and cimetidine presented an $AUC_{0–240\ min}$ of 9.61 ± 0.9 mL min. Cyproheptadine reduced edema from the first 10 min of analysis ($AUC_{0–240\ min}$ of 2.76 ± 0.3 mL min) (Fig. 3c). L-NAME ($AUC_{0–240\ min}$ of 5.26 ± 1.0 mL min) had a similar effect as

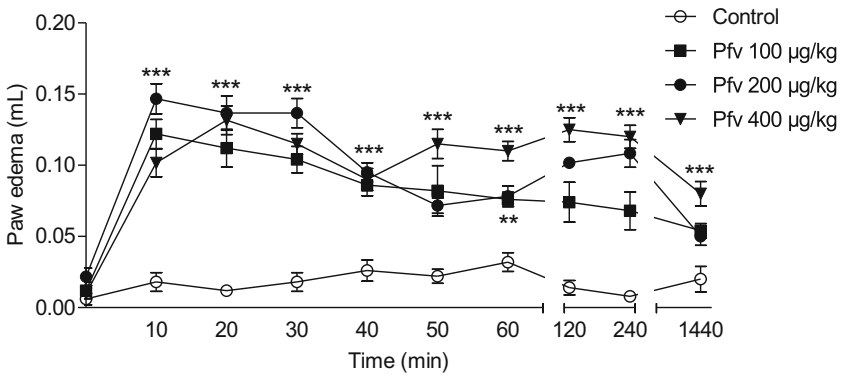

Fig. 1. Time course of the paw edema induced by *Parachartergus fraternus* venom (Pfv) in mice. Values are expressed as the mean ± SEM ($n = 7$). Two-way ANOVA was performed followed by Bonferroni test. All doses in the analyzed periods induced edema with $p < 0.001$ (***), except for 60 min. **$p < 0.01$ ***$p < 0.001$ compared with the control group (saline).

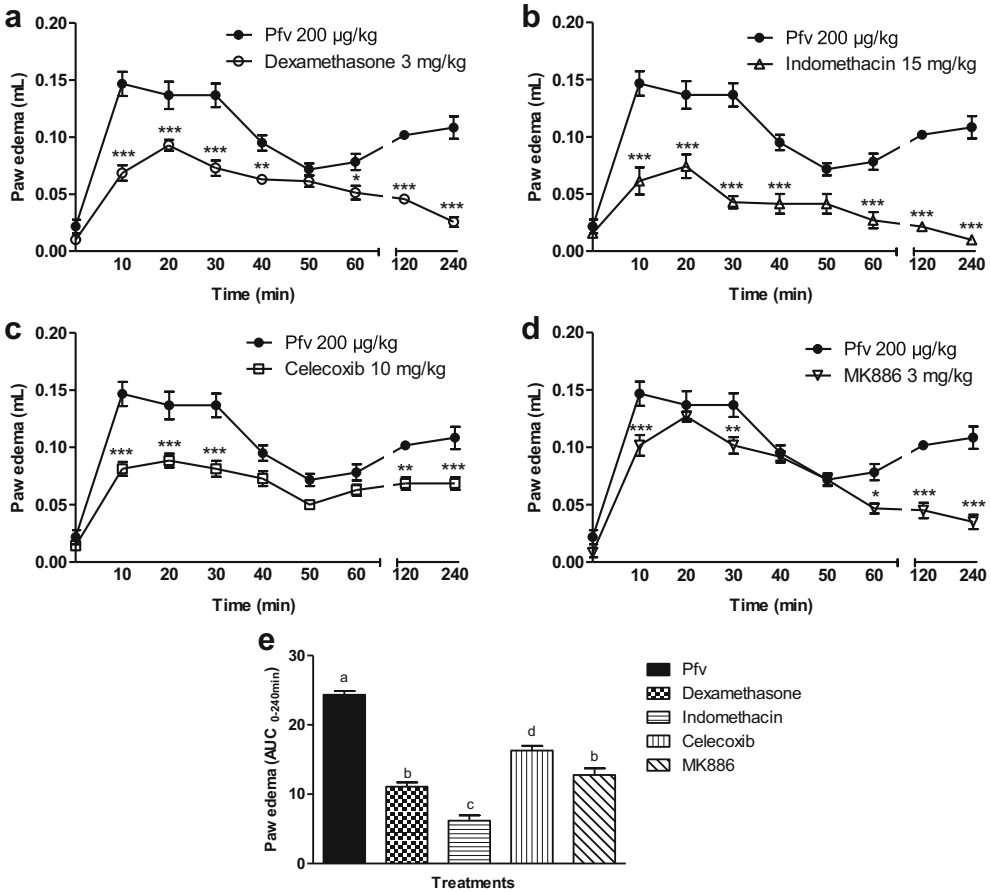

Fig. 2. Effect of inhibiting enzymes involved in the arachidonic acid pathway on the paw edema induced by *Parachartergus fraternus* venom (Pfv). The animals were pretreated with dexamethasone, a FLA_2 inhibitor (**a**); indomethacin, a non-selective COX inhibitor (**b**), celecoxib, a COX-2 inhibitor (**c**), or MK886, a leukotriene synthesis inhibitor (**d**), and the area under the curve (AUC) was calculated for each of the drugs from 0 to 240 min (**e**). Values are expressed as the mean ± SEM ($n = 7$). Two-way ANOVA was performed followed by Bonferroni test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the Pfv group. Different letters indicate statistically significant differences.

cyproheptadine (Fig. 3e), while the $AUC_{0–240\ min}$ of captopril was 12.1 ± 0.9 mL min.

DISCUSSION

Edema is a frequent symptom after wasp and bee stings, affecting more than 80% of sting victims [21], and the anaphylactic shock associated with swelling at the site of the sting can lead to airway closure, resulting in deleterious effects on the victim and potentially death [10].

The analysis of the edematogenic kinetics of Pfv showed that the peak of the development of paw edema in mice was between 10 and 30 min after the administration of a dose of 200 µg/kg (equivalent to 5 µg/paw). This effect persisted for up to 240 min, except for the dose of 400 µg/kg that maintained the effect for 24 h. Mortari et al. [11] observed the edematogenic effect of Pfv on rats for a period of up to 60 min. However, our study went further, using another animal species and an extended period of evaluation. Data show that this event is induced in different species with varying inoculated kinetics and quantity, which may interfere with symptomatic treatment since mediators are not necessarily released in the same sequence and at similar concentrations [21].

Similarly, previous studies have shown that the venom of *Polybia* species induced edema in rats, and the peak of the edema induced with 40 µg/paw occurred 20 min (*Polybia paulista*), 30 min (*Polybia occidentalis*), and 40 min (*Polybia ignobilis*) after the injection [22]. On the

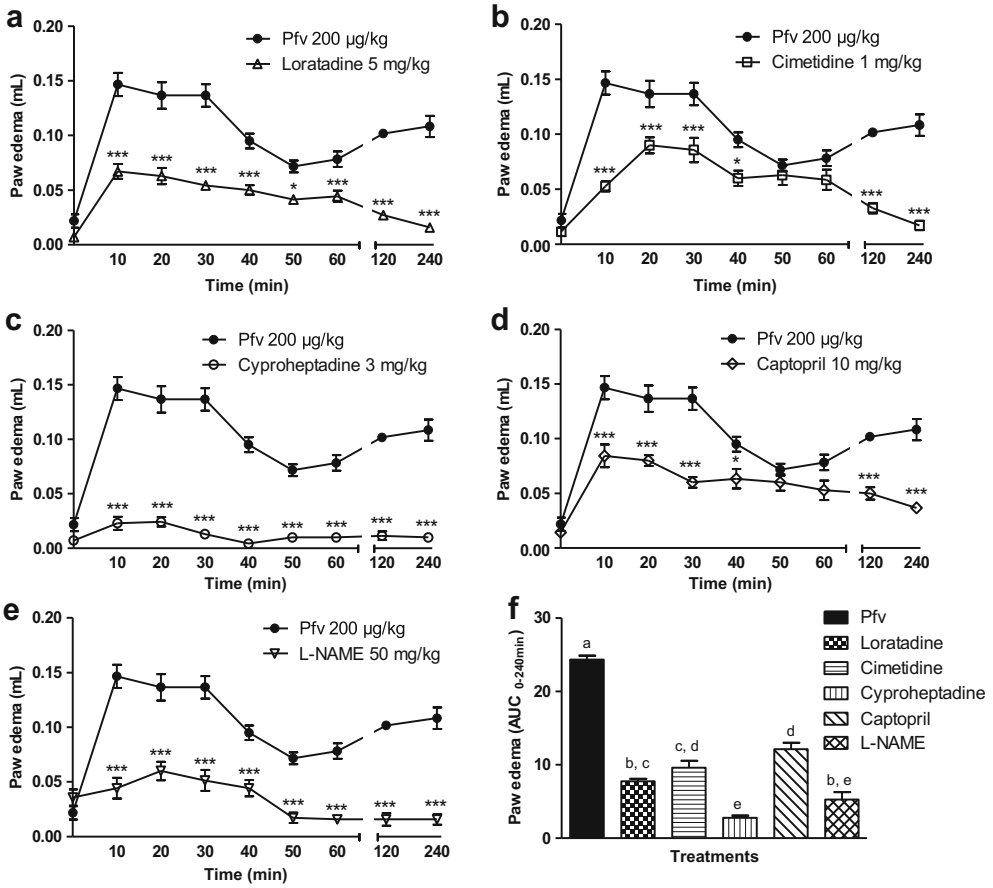

Fig. 3. Effect of vasoactive amine antagonists, an angiotensin-converting enzyme (ACE) inhibitor and nitric oxide synthase (NOS) inhibitor, on the paw edema induced by *Parachartergus fraternus* venom (Pfv). The animals were pretreated with loratadine, a histamine H_1 receptor antagonist (**a**); cimetidine, a histamine H_2 receptor antagonist (**b**); cyproheptadine, a dual antagonist of histamine H_1 and serotonin 5-HT receptors (**c**); captopril, an inhibitor of ACE (**d**); L-NAME, a nonselective inhibitor of NOS (**e**); and the area under the curve (AUC) for each of the drugs was calculated from 0 to 240 min (**f**). Values are expressed as the mean ± SEM ($n = 7$). Two-way ANOVA was performed followed by Bonferroni test. $^{*}p < 0.05$ and $^{***}p < 0.001$ compared with the Pfv group. Different letters indicate statistically significant differences.

other hand, the venom of the wasp *Polistes lanio lanio* induced this effect in mice 120 min (peak action) after the injection of 7 µg/paw [23].

The differences in the dose and time may be due to the composition of the venoms, specifically the type and quantity of biogenic amines, proteins, and peptides [24, 25]. Biogenic amines have previously been identified in Pfv (unpublished data), and Gonçalves et al. [26] showed the presence of the mastoparan peptide Agelaia-MPI in Pfv. These venom constituents also degranulate the mast cells and can amplify the edematogenic response in coordination with the mediators released by the victim immediately after the sting [24, 27, 28].

Considering the pathways and mediators possibly involved in the edematogenic response, we observed that components of the arachidonic acid (AA) pathway, vasoactive amines, and nitric oxide (NO) are involved in the edema development induced by Pfv.

Initially, we analyzed the AA pathway. It is known that COX acts in the oxidation of AA, resulting in the subsequent synthesis of prostaglandins (PG) and thromboxanes (TX), and that PG potentiates edema formation [29, 30]. Our results showed that PGs act in this event 30 min after Pfv injection, since this period was the peak of indomethacin and celecoxib action, which predominantly inhibits the synthesis of PGs by the action of COX-1 and COX-2, respectively. Although COX-1 is a constitutive enzyme, studies have reported the expression of this enzyme in inflammatory processes [31, 32], which corroborates our findings that indomethacin reduced the edema.

Other authors, in assays investigating the venoms of the wasps *P. paulista* [16] and *Polistes fuscatus* [24], did not observe this inhibitory effect with indomethacin, which may be due to differences in the protein components such as phospholipases that act on lysis of membrane phospholipids with release of AA or in the type of peptides present as mastoparans [25, 33].

MK886 presented a low potential to reduce edema (31%); it was effective only beginning at the second hour after Pfv inoculation, confirming that the cysteinyl leukotrienes LTC4, LTD4, LTE4, and LTF4 participate in the late stages of this process, considering that these mediators participate in the formation of edema by increasing vascular permeability [34].

Dexamethasone, a drug derived from glucocorticoid hormones, reduced the edema observed at 10 min after Pfv injection, and this effect was maintained throughout the analyzed period. This inhibition was on average 40%, which differed from the inhibition observed with another species of the order Hymenoptera, *Dinoponera quadriceps*, in which dexamethasone inhibited more than 90% of the mice paw edema induced by the venom of this ant [35]. Glucocorticoids inhibit phospholipase A2 (PLA2) [36], inhibit the expression of pro-inflammatory cytokines such as TNF and IL-1, and synthesize and release anti-inflammatory mediators such as annexins [37, 38]. Furthermore, glucocorticoids inhibit the expression of COX-2 and inducible NOS [39]. These agents are considered the anti-inflammatory drug of choice and are used in addition to antihistamines in the treatment of victims of wasp envenomation [40].

Thus, considering the AA pathway, we observed that indomethacin presented a lower AUC than dexamethasone, whereas MK886 and celecoxib had the highest AUCs. Therefore, the former was more effective than the other drugs at reducing edema, which indicates the prominent participation of COX, in particular COX-1, in the formation of edema. In addition to lipid mediators resulting from AA metabolism, vasoactive inflammatory mediators such as histamine, serotonin, bradykinin, and NO can induce edema formation [41, 42].

Our results showed that H_1 and H_2 receptors are involved in the Pfv-induced formation of edema since the antihistamines loratadine and cimetidine both effectively reduced edema beginning at 10 min after the injection of Pfv.

Loratadine maintained a constant reduction in edema throughout the experimental period, with a lower AUC than cimetidine, indicating the increased participation of the H_1 receptor in this inflammatory event. In this work, we evaluated the participation of both receptors; however, other authors previously identified two other types of histamine receptors, H_3 and H_4, which may also be involved in the formation of edema [43, 44] but were not evaluated at the time.

The involvement of histamine and serotonin in the effect induced by Pfv was confirmed by the use of cyproheptadine, which blocks histamine H_1 and serotonin 5-HT receptors, negatively modulating edema, and other events that may occur following wasp stings such as urticaria and allergic reactions [45]. We emphasize that this drug was the most effective in reducing edema throughout the analyzed period.

These data indicate that histamine and serotonin are the main mediators responsible for the formation and maintenance of edema caused by Pfv. The fractionation of this venom identified the presence of biogenic amines in the venom (unpublished data). These amines, associated with the histamine and serotonin released by the degranulation of mast cells [46] at the moment of the sting, act together to maintain the inflammatory effect induced by different species of wasps [16, 23]. Furthermore, antiserotonergic drugs more effectively reduced the edema induced by the wasps *P. fuscatus* [24] and *Vespula vulgaris* [28].

Another vasoactive inflammatory mediator that we evaluated was bradykinin. We used the drug captopril, an ACE inhibitor, because when ACE is inhibited, bradykinin accumulates [47], which could potentiate edema. However, to our surprise, we observed a reduction in this effect. Such response may have occurred by the i.p. administration of captopril, since it has already been shown to suppress the vascular permeability induced by histamine, serotonin, bradykinin, and compound 48/80 [48], in addition to inhibiting mast cell degranulation [49]. The subcutaneous injection of captopril also reduced the edema caused by the injection of venom from other venomous animals [24, 50]. Despite the increase in bradykinin after captopril administration, there is no potentiation of edema due to a systemic inhibition of mast cell degranulation, preventing the release of mediators that act in the formation of edema.

In addition, it was evaluated the participation of NO, a potent vasodilator, in envenoming can act in the formation of edema [41]. This inflammatory mediator can be synthesized by induced or constitutive NOS [51], and to view its role in the formation of edema, the NOS inhibitor, L-NAME, is used. L-NAME reduced edema by approximately 70% after 10 min, and its efficacy was inferior only to cyproheptadine, indicating the important participation of this mediator in the formation of edema. Our results corroborate those observed with other species of Hymenoptera, such as ants [35] and bees [41].

Therefore, the results obtained with the venom of the *P. fraternus* wasp indicate that the peak of paw edema occurred at 10 min with a dose of 200 μg/kg. The evaluation of the pathways and mediators involved indicates that the AA pathway and the inflammatory mediators histamine, serotonin, and NO participate in the formation of edema. The drugs of choice used in the treatment of victims are glucocorticoids and antihistamines [40]; however, cyproheptadine was the drug that most effectively reduced edema, which indicates that a change in the adopted therapy could result in increased treatment success and could minimize the time and costs required for the treatment of *P. fraternus* wasp stings.

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COMPLIANCE WITH ETHICAL STANDARDS

All experiments were approved by the Ethics Committee on Animal Experimentation of the UFMS (protocol 764/2016) and were conducted in accordance with the National Institutes of Health Regulations on the Use and Care of Animals for Scientific Purposes.

Conflict of Interest. The authors declare that they have no conflict of interest.

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