



Effect of *Rhopalurus junceus* Scorpion Venom on Inflammation-Related Cytokines in Healthy BALB/C Mice

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Abstract

Introduction: Scorpion venoms significantly increases the production of pro-inflammatory cytokines that contributes to immunological imbalance. However, there is no evidence about the capabilities of Cuban scorpion *Rhopalurus junceus* (*R. junceus*) venom to modulate the levels of inflammation-related cytokines.

Methods: One scorpion venom dose (3.2 mg/kg) was administrated during one or ten days by intraperitoneal route to BALB/c healthy mice. The pro-inflammatory (TNF α , IL-1 β , IL-12) and anti-inflammatory (IL-10) cytokines levels in sera were determined by ELISA after 2, 4, 6, 8 and 24 hours for the unique or the last injection of multiple scorpion venom administration. Kruskal-Wallis's test was used for different time period comparison.

Results: Administration of a single dose of *R. junceus* scorpion venom does not increase the levels of pro-inflammatory cytokines. While IL-10 level increase significantly ($p < 0.05$) only after 4 hours of single dose administration of scorpion venom. Additionally, there were no changes in pro- and anti-inflammatory cytokine levels, at different times evaluated after 10 consecutive administration of scorpion venom dose.

Conclusion: *R. junceus* scorpion venom does not cause an imbalance between pro- and anti-inflammatory cytokine levels in healthy BALB/c mice, unlike other scorpions belonging to the *Buthidae* family.

Keywords: *Rhopalurus junceus* scorpion venom; Inflammation-related cytokines; Mice; ELISA

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Introduction

Scorpion venoms consist of a complex of several toxins that exhibit a wide range of biological properties and actions, as well as chemical compositions, toxicity, pharmacokinetic and pharmacodynamic characteristics [1]. The *Buthidae* family comprises scorpion species that present medical importance considered a public health problem with frequent statements that scorpion stings are dangerous [2]. Accidents caused by scorpion stings are a relatively common event in subtropical and tropical countries and can cause lethal envenomation in humans, especially in children. The signs of the scorpion envenomation are determined by the following: scorpion species, venom composition and the victim's physiological reaction to the venom [3]. Although the pathophysiology of envenomation is complex and not yet fully understood, venom and immune responses are known to trigger the release of inflammatory mediators that are largely mediated by cytokines [3]. The activation and release of cytokines may play an important role in the pathophysiology of envenomation after stings of scorpion from *Buthidae* family, which are responsible for some systemic inflammatory manifestations and organ failure [4,5]. In case of *Leiurus quinquestratus*, belonging to *Buthidae* family, has been reported that there is a correlation between the overproduction of pro-inflammatory cytokines as the IL-1 with symptoms associated to the scorpion envenomation [6]. Another pro-inflammatory cytokine that is increased after scorpion sting is TNF- α . Razi et al observed that rats stung by the scorpion *Mesobuthus Eupeus* increase significantly the TNF- α levels in serum and this correlate positively with the clinical severity of symptoms [7].

The scorpion *Rhopalurus junceus* (*R. junceus*) is endemic species from Cuba belonging to *Buthidae* family. Its venom has been used as a popular treatment in traditional medicine. Besides, there are no reports of scorpionism from this species in the country, for this reason, it is not considered dangerous to humans [8]. However, until now, there is no scientific evidence about the capabilities of *R. junceus* venom to modulate the levels of inflammation-related cytokines. Due this, the purpose

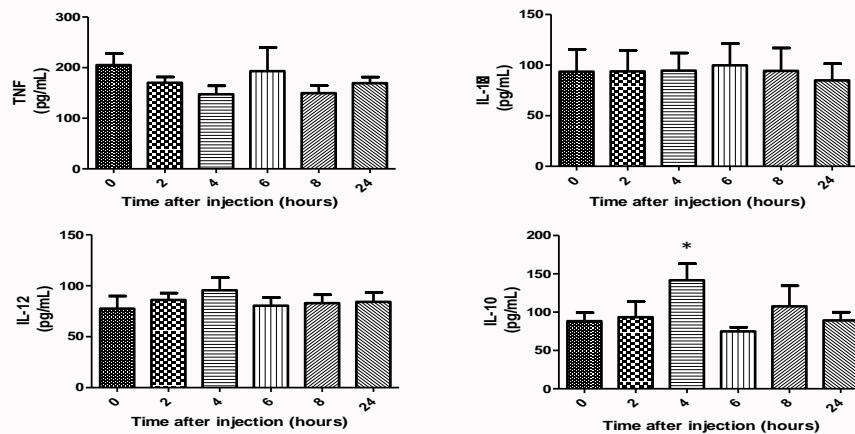


Figure 1: Kinetic of cytokines levels induced by intraperitoneal injection after one injection of 3.2 mg/kg of *R.junceus* scorpion venom. Each bar represents the mean value \pm SD from two independent experiments conducted with five animals each. Statistical differences between treatments were marked with asterisk ($p < 0.05$).

of this work is to study the balance of pro and anti-inflammatory cytokines induced by administration of *R. junceus* scorpion venom in healthy mice.

Materials and Methods

Venom source

Rhopalurus junceus scorpions were maintained in individual plastic cage in the laboratories belonging to the Laboratories of Biopharmaceuticals and Chemistries Productions (LABIOFAM). Venom was obtained by electrical stimulation of scorpion telson. Venom was dissolved in distilled water, centrifuged and supernatant was filtered by 0.2 μ m syringe filter. The protein content was calculated by the method of Lowry modified [9].

Animals

BALB/c male mice with an average weight of 20 ± 2 g were obtained from the National Center for Laboratory Animal Breeding (CENPALAB, Havana, Cuba). Mice were housed in controlled temperature and humidity rooms and food and water were administered ad libitum. The experimental procedures using animals were approved by the Institutional Committee for the Care and Use of Laboratory Animals (Protocol 2013/3) and performed in accordance with the International Guiding Principles for Biomedical Research Involving Animals.

Measurements of cytokines level

Two groups of 5 mice each were injected by intraperitoneal route with the scorpion venom dose of 3.2 mg/kg dissolved in 0.1 ml of saline buffer. One group was administered with a unique dose and the second group was administered with ten consecutive injections during ten days. Two control mice group received 0.1 ml of saline buffer with the same procedure of scorpion venom-treated groups. Mice were bled after 2, 4, 6, 8 and 24 hours of the unique or the last injection from multiple scorpion venom administrations, and sera were separated and stored at -20°C until use. The pro-inflammatory (TNF α , IL-1 β , IL-12) and anti-inflammatory (IL-10) cytokines levels in sera were determined by an enzyme-linked immunosorbent assay using a set of monoclonal antibodies (BD OptEIA™; BD Biosciences, San Diego, CA, USA). The procedure was performed according to the manufacturer's instructions.

Statistical analysis

Results are presented as the mean \pm Standard Deviations (SD). Statistical analysis was performed by Kruskal-Wallis's test, post-tests: Dunn, using GraphPad Prism version 5.01 for Windows, (GraphPad Software, San Diego California, USA). Significant differences were considered for $p < 0.05$.

Results

BALB/c mice were injected intraperitoneally once or ten consecutive times with 3.2 mg/kg of *R. junceus* scorpion venom. Figure 1 shows the effect of the scorpion venom after single dose injection. There are no statistically significant differences between TNF, IL-1 β and IL-12 levels in the scorpion venom-treated group compared to control group (Figure 1A-C). However, a significant increase ($p < 0.05$) of IL-10 was observed 4 hours after the injection that subsequently decays (Figure 1D). For the rest of the evaluated times, IL-10 levels do not differ respect the control group. Additionally, when ten doses of scorpion venom were administered, there were not observed statistically significant differences at 2, 4, 6, 8 and 24 hours after the last injection in the scorpion venom-treated group compared to control group (Figure 2A-D).

Discussion

R. junceus scorpion venom is endemic specie from Cuba belonging to the *Buthidae* family. There are no reports of scorpionism from this specie in our country. However, there is no scientific information about its effect on inflammation-related cytokines.

In this work, we demonstrate that after *R. junceus* venom injection, only IL-10 showed a transient increase after one dose injection treatment. The remaining pro-inflammatory cytokines in both treatment regimens do not showed variation after venom injection. These results differ from dangerous scorpion species belonging to *Buthidae* family which usually provoke cytokines imbalance as part of human scorpionism [1]. One example represents *Centruroides noxius* scorpion venom which provokes an increase of inflammation-related cytokines such as: IL-6, IFN- γ and IL-10 in mice compared to those from non-injected groups after subcutaneous administration [4]. In rats, administration of *Androctonus australis hector* scorpion venom increased the concentration of IL-1 β , IL-4, IL-6, IL-10, TNF- α , IL-5 and IL-12 [10,11]. Besides, high levels of pro and/or anti-

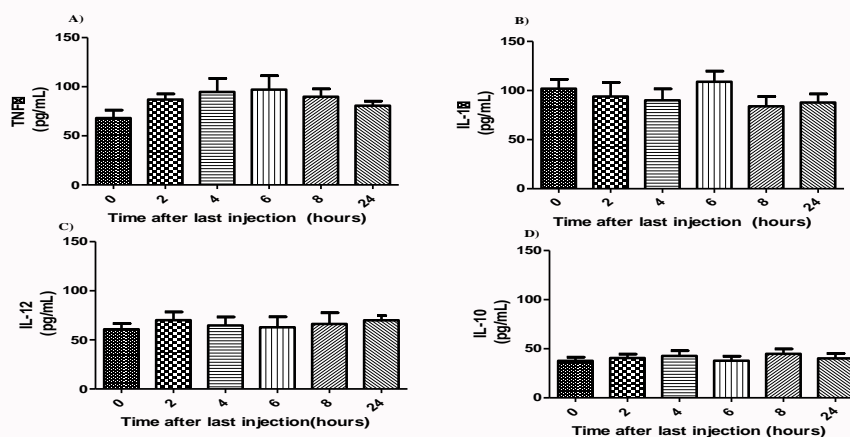


Figure 2: Kinetic of cytokines levels induced by intraperitoneal injection after ten consecutive injections of 3.2 mg/kg of *R. junceus* scorpion venom. Each bar represents the mean value \pm SD from two independent experiments conducted with five animals each.

inflammatory cytokines (IL-1 β , IL-6, IL-8, IL-10, NO, TNF- α , IL-1 α , IFN- γ , and GM-CSF) were observed in human and mice after *Tityus serrulatus* (Tsv) scorpion venom sting [12,13].

Scorpion venoms from *Buthidae* family induce severe systemic inflammation that involve cytokine imbalance, multiple organ dysfunction and death, after injection of lethal doses usually under 2 mg/kg [14]. The dose selected in the study has been tested for acute toxicity and was not observed toxic symptom in male BALB/c mice (manuscript in preparation). In another study, *R. junceus* scorpion venom doses (2.5 mg/kg-10 mg/kg) were evaluated in healthy CD-1 mice and only was observed slight discomfort at the highest dose [15]. Despite of high dose used in this study, *R. junceus* scorpion venom does not cause an imbalance between pro-and anti-inflammatory cytokine levels in healthy BALB/c mice, unlike other scorpions belonging to the *Buthidae* family. All these evidences suggest a low potential of *R. junceus* venom to induce sign and symptoms associated to envenomation process.

Conclusion

R. junceus scorpion venom is not able to modulate the levels of inflammation-related cytokines which are very important mediators of envenomation process.

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