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Venom's antinociceptive property in the primitive ant *Dinoponera quadriceps*

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ABSTRACT

Ethnopharmacological relevance: In northeastern Brazil, *Dinoponera* (Ponerinae) ants macerate are used to treat ear ache and its sting, rheumatism, and back pain. Such a popular use is a relevant fact that called for experimental evaluation of the antinociceptive activity of *Dinoponera* venom.
Materials and methods: Dinoponera quadriceps venom (DqV; 5–500 μg/kg; i.v.) or morphine (3.4 mg/kg; s.c.) were evaluated in mice models of nociception ($n=8$ animals/group). Negative controls received sterile saline (0.9% NaCl; i.v.).
Results: DqV showed 64% protein content and exhibited antinociceptive activity, without affecting motor function, in the tests: formalin (72%), writhing (52%), von Frey (71%) and hot plate (45%). The antinociceptive activity was abolished under protein denaturant conditions.
Conclusions: This study provided the first demonstration of the antinociceptive property of *Dinoponera quadriceps* venom in mice models of chemical, mechanical and thermal nociception, corroborating the popular use and suggesting its potential therapeutic utilization in painful conditions.

1. Introduction

Dinoponera (Ponerinae) is a primitive and strictly Neotropical ant genus, with six known species considered the biggest ants of the world (3–4 cm in length), (Kempf, 1971). Similar to the other *Dinoponera* species, *Dinoponera quadriceps* Santschi, 1921 ("falsa tocandira", "trinca-cunhão"), has ground-dwelling habits and their common prey are medium size to large arthropods that they subdue with their sting (Araújo and Rodrigues, 2006). Like many ants of basal and more derived groups (*i.e.* Ponerinae, Pseudomyrmeciinae, Myrmicinae), as well as other hymenopteran species (bees and wasps), *Dinoponera* ants have a sting apparatus, located in the last portion of the gaster, formed by the sting itself along with two associated glands: Dufour's gland and venom gland. In ants and other hymenopterans, the main function of venom is prey capture and/or defense (Buschinger and Maschwitz, 1984; Schmidt, 1986).

Primitively, the composition of hymenopteran venoms is a complex mixture of biologically active proteins and other proteinaceous elements (Schmidt, 1986). The venom of *Paraponera clavata* (Ponerinae) contains a peptide (poneratoxin) that blocks sodium channels in frog skeletal muscle fibers (Duval et al., 1992). Some hymenopteran protein venom components from *Apis mellifera* (melittin) (Merlo et al., 2011) and from the social wasp *Polybia occidentalis* (Thr^6-bradykinin) exhibit antinociceptive properties (Mortari et al., 2007). Few studies have explored the use of ant venoms in the folk medicine. However, there is a description of the popular use of *Dinoponera* sp. for earache in Ichu-Bahia (northeastern Brazil): the product obtained from crushed ants is applied in the ear using a piece of cotton (Costa Neto, 2011). *Dinoponera* sp. sting is also said to be useful to treat rheumatism and back pain (Costa Neto et al., 2006). In addition, there are descriptions of *Dinoponera* sp. popular use for asthma treatment (Costa Neto et al., 2006; Alves and Rosa, 2007). The aim of this study is to evaluate the antinociceptive property of *Dinoponera quadriceps* venom in mice.

2. Materials and methods

2.1. Materials

Dinoponera quadriceps nests were collected (IBAMA authorization no. 28794-1) in the "Serra de Maranguape", a small mountain range located in the littoral zone of the Ceará state (northeastern Brazil), and kept in plastic boxes (63 cm × 42 cm and 12 cm high), at 30 ± 2 °C, with a 12/12 h light/dark cycle, and PVC tubes (4 cm in diameter, 38 cm in length) as nesting sites. Ants were fed *ad libitum* on *Tenebrio molitor* larvae. To collect the venom,

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Dinoponera quadriceps workers were seized in the thorax region and its sting was introduced in a capillar tube to induce venom secretion. Venom was then transferred to a tube containing 10 mM ammonium acetate buffer (pH 6.8), lyophilized and stored at −20 °C. Protein content was evaluated by the colorimetric method ($A_{595\ nm}$).

Male Swiss mice (25–35 g), maintained at 25 °C under a 12/12 h light/dark cycle with food and water *ad libitum,* were brought to the laboratory at least 1 h before the experiments, approved by the Animal Care and Use Committee (UECE—11221997-7/45).

Drugs and reagents were purchased from Sigma, St. Louis, MO, USA (λ-carrageenan), Nova Química, São Paulo, SP, Brazil (Diazepan), Isofar, Rio de Janeiro, RJ, Brazil (formaldehyde and acetic acid).

2.2. Nociception models

Mice (n=8 animals/group) received *Dinoponera quadriceps* venom (DqV; 5–500 µg/kg) by intravenous (i.v.) route or morphine (3.8 mg/kg) by subcutaneous (s.c.) route 30 min before injection of the nociceptive agents. Negative controls received sterile saline (0.9% NaCl; 50 µL/10 g body weight; i.v.).

Formalin test: formalin (2.5% v/v; 20 µL/paw) was injected by subcutaneous (s.c.) intraplantar route in the animal right hind paws and the time (s) in which they spent licking their paws was registered during the first (P1: 0–5 min) and second (P2: 15–30 min) phases of the test (Shibata et al., 1989).

Writhing test: acetic acid (0.8% v/v; 0.1 mL/10 g body weight) was injected by intraperitoneal (i.p.) route and the number of abdominal writhes was registered during 10–30 min (Koster et al., 1959).

von Frey test: animals were individually placed in clear acrylic boxes with raised platforms of wire mesh to allow access to the ventral surface of hind paws from 15 to 30 min. Hypernociception was induced by s.c. injection of 1% carrageenan (300 µg/paw) and the frequency of paw withdrawal in response to six applications of the flexible von Frey filament (0.8 g) was measured before (*T*0) and from 1 to 5 h after carrageenan (von Frey, 1896).

Hot plate test: mice were placed on a hot plate at 55 ± 0.5 °C for up to 25 s and the reaction latency of thermal stimulus (time to start licking or shaking hind paws or jumping) was registered. Treatment with DqV at r.t. or at 100 °C/1 h was performed before the test. The reaction latency was recorded at baseline and after 1–5 h (Hunskaar et al., 1986).

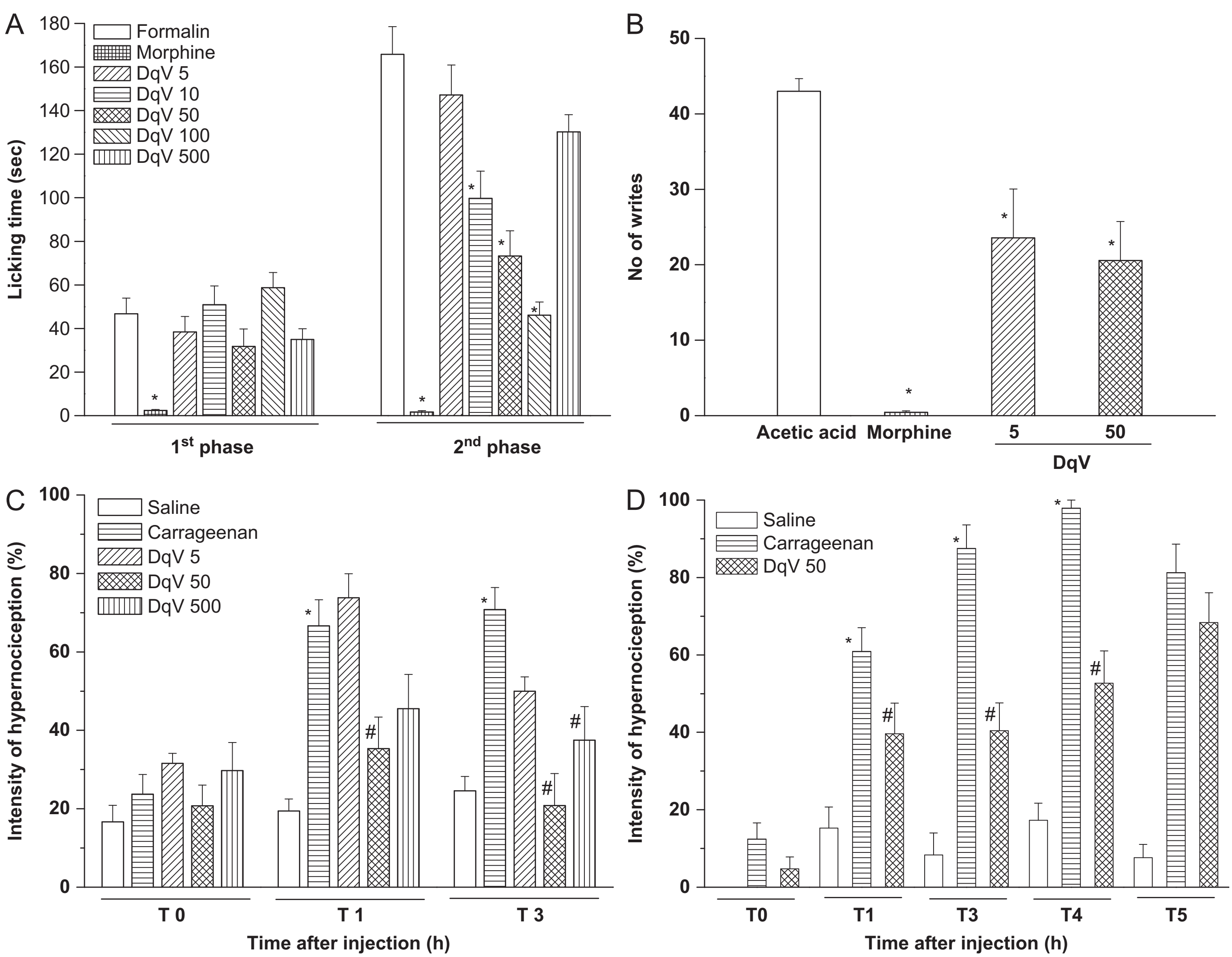

Fig. 1. *Inhibitory effect of DqV in models of chemical and mechanical nociception*. Mice were treated with DqV (5, 10, 50, 100 and 500 µg/kg; i.v.) or morphine (3.8 mg/kg, s.c.) 30 min before nociceptive stimuli: (A) Formalin (2.5% v/v; 20 µL; s.c.), (B) acetic acid (0.8% v/v; 0.1 mL/10 g body weigh; i.p.) (C, D) von Frey test (1% carrageenan; 300 µg/paw; s.c.). Mean ± SEM (n=8). ANOVA and Bonferroni's test. *$p<0.05$ *vs.* formalin, acetic acid or saline. #$p<0.05$ *vs.* carrageenan.