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Evaluation of the antiedematogenic activity of artemetin isolated from *Cordia curassavica* DC

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Abstract

The species *Cordia curassavica* (Boraginaceae), known popularly as "erva baleeira", is used in folk medicine for the treatment of several inflammatory processes and as a healing agent. The objective of the present study was to evaluate the antiedematogenic activity of crude dichloromethane extracts of *Cordia curassavica* and of the artemetin-enriched fraction. The crude extract and artemetin fraction were tested in the model of carrageenin-induced paw edema in male Swiss mice (25-30 g). The crude dichloromethane extract (300 and 1000 mg/kg, *po*, N = 6) showed significant antiedematogenic activity, reducing the edema by 42, 57 and 45% and 46, 62 and 69%, 3, 4 and 5 h after carrageenin administration, respectively. Indomethacin (10 mg/kg, *po*, N = 6) reduced the edema by 45 and 48%, after 4 and 5 h, but the artemetin-enriched fraction (30, 100 and 300 mg/kg, *po*, N = 6) had no activity. The dichloromethane extract (300 and 1000 mg/kg, *po*, N = 6) also showed antinociceptive activity by reducing acetic acid-induced writhing in mice from 37.1 ± 2.28 (control) to 17.3 ± 1.34 and 13.2 ± 1.44, respectively, but had no activity in the hot-plate test.

Key words
- *Cordia curassavica*
- *Cordia verbenaceae*
- Antiedematogenic
- Anti-inflammatory
- Medicinal plants

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The species of the genus *Cordia* (Boraginaceae) are found throughout the tropical and subtropical areas of Asia, Africa, Australia, Guyana and South America (1). Known popularly in Brazil as "erva baleeira", *Cordia curassavica* was previously classified as *C. verbenaceae*, and occurs in Brazil from the Amazonian region to Rio Grande do Sul, preferentially at a distance of 500 to 1000 m from the seashore (2). The crude ethanol extract of the herb's aerial parts (leaves and stems) is widely used by the seashore population to treat several inflammatory processes and is generally applied topically to the afflicted areas. Some investigators have suggested that the flavonoid artemetin is the compound responsible for the anti-inflammatory activity of this species (3). The crude ethanol extract reduced the paw edema induced by carrageenin in a dose-dependent manner (0.59 to 2.98 mg/kg, with ED_{50} of 1.24 mg/kg) (4). However, the flavonoids isolated from the crude extract were active only at doses of 30.4 to 153.9 mg/kg, with ED_{50} of 67.07 mg/kg (3). These apparently contradictory results suggest that artemetin is not the main substance responsible for the anti-inflammatory activity of crude *Cordia* extracts, since the activity of the pure compound was 54 times lower than that observed

for the crude extract.

As a result of these considerations, we evaluated the antiedematogenic activity of several crude *C. curassavica* extracts and of an enriched 5-hydroxy-3,6,7,3'4'-penta methoxyflavone (artemetin) fraction.

Leaves and stems of *C. curassavica* were collected from the experimental field of Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA) of Unicamp. A voucher specimen is deposited at the Biological Institute of Unicamp under the reference number UEC 112744. The material was allowed to dry under circulating air (40ºC) and ground. The powder was submitted to dynamic maceration with different solvents (petroleum ether, dichloromethane, ethanol) for 4-h periods. This procedure was repeated three times with each solvent. Concentration of each extract under reduced pressure yielded extracts denoted petroleum ether, dichloromethane and ethanol crude extracts.

Artemetin was isolated from the crude acetone extract of *C. curassavica*. The compound was isolated by column chromatography with silica gel using hexane/ethyl acetate gradients. The fraction containing artemetin was crystallized in pentane, yielding yellow needles with a melting point of 154-155ºC. The structure of artemetin was confirmed by comparison of hydrogen nuclear magnetic resonance (H'NMR) and ^{13}C NMR experimental data with those reported in the literature (5).

Pharmacological activity was evaluated in male Swiss mice weighing 25-30 g from the University animal facilities. The animals were used after at least seven days of adaptation to the experimental environment, with 12-h light-dark cycles and room temperature of 25 ± 3ºC.

The antiedematogenic activity was evaluated using the carrageenin-induced mouse paw inflammation model (6). In this experiment, six male mice were used in each group. Group I, negative control, was treated with vehicle (2% polysorbate in 0.9% NaCl, 10 ml/kg), groups II-IV received the crude petroleum ether, dichloromethane and ethanol extracts, respectively (1000 mg/kg, *po*), and group V was treated with indomethacin (10 mg/kg, *po*) as positive control. One hour after administration, edema was induced in the animals by injecting 0.05 ml of 1% (w/v) carrageenin in normal saline into the plantar aponeurosis of the left hind paw. Paw volume was measured with an Ugo Basile Plethysmograph model 7150 at time zero (immediately after carrageenin injection) and 30, 60, 120, 180, 240 and 300 min after the injection (6). The results are reported as mean ± SEM and the individual data were submitted to one-way analysis of variance followed by the Duncan test, with the level of significance set at $P<0.05$ for both tests.

The dichloromethane extract (1000 mg/kg) reduced the edema by 59 and 68%, 4 and 5 h after carrageenin administration, respectively, whereas the ethanol extract (1000 mg/kg) reduced edema by 44% after 4 h. Indomethacin (10 mg/kg), used as standard, reduced the edema by 45 and 44%, 4 and 5 h after carrageenin administration, respectively. The crude petroleum ether extract (1000 mg/kg) did not produce a significant reduction in paw edema.

The crude dichloromethane extract and artemetin fraction were used to study the relationship between dose and response in the edema model. Six male mice were used in each group. Group I, negative control, was treated with vehicle (2% polysorbate in 0.9% NaCl, 10 ml/kg) and groups II-IV received the crude dichloromethane extract (100, 300 and 1000 mg/kg, *po*). The artemetin fraction, was tested similarly using 30, 100 and 300 mg/kg, *po*, and group V was treated with indomethacin (10 mg/kg, *po*) as positive control.

The dichloromethane extract (300 mg/kg) reduced the edema by 42, 57 and 45%, 3, 4 and 5 h after carrageenin administration, respectively, whereas the highest dose (1000

mg/kg) reduced the edema by 46, 62 and 69% after 3, 4 and 5 h, respectively. Indomethacin (10 mg/kg), used as standard, reduced the edema by 45 and 48%, 4 and 5 h after carrageenin administration, respectively (Figure 1). The artemetin-enriched fraction did not produce significant paw edema reduction at doses of 30, 100 or 300 mg/kg (Figure 2).

The results of the present study demonstrate that the antiedematogenic substances present in *C. curassavica* are mainly extracted by medium polarity solvents, since paw edema reduction was obtained with dichloromethane and ethanol extracts. Artemetin was detected in the extracts and, according to Sertié et al. (3), the presence of this flavonoid would justify their antiedematogenic activity. However, our results demonstrated that the purified artemetin fraction did not present any antiedematogenic activity at doses up to 300 mg/kg in the experimental model used when administered orally.

Flavonoids are a group of about 4000 substances widely distributed in nature. The human daily diet contains approximately 1 g of flavonoids. Flavonoids have anti-inflammatory, analgesic, anticancer, antihepatotoxic, antiulcer, antioxidant and antispasmodic activities (7). However, most of these flavonoids are degraded in the digestive system (8,9). This can explain the absence of an antiedematogenic effect observed with artemetin even at doses of 300 mg/kg. However, this cannot be tested directly because intraperitoneal, subcutaneous or intramuscular administration of artemetin provokes a local inflammatory process which interferes with the assay.

Previous studies on the *Cordia* ethanol extract have revealed anti-inflammatory activity at doses of 0.6 to 3.0 mg/kg (3) with topical administration (10). The anti-inflammatory potency of the crude extract was comparable to that of pure steroids such as dexamethasone. Since it is not very probable that a crude extract that should contain hundreds of substances can have such high potency, it is likely that the result was due to the presence of solvents and or the release of endogenous corticoids.

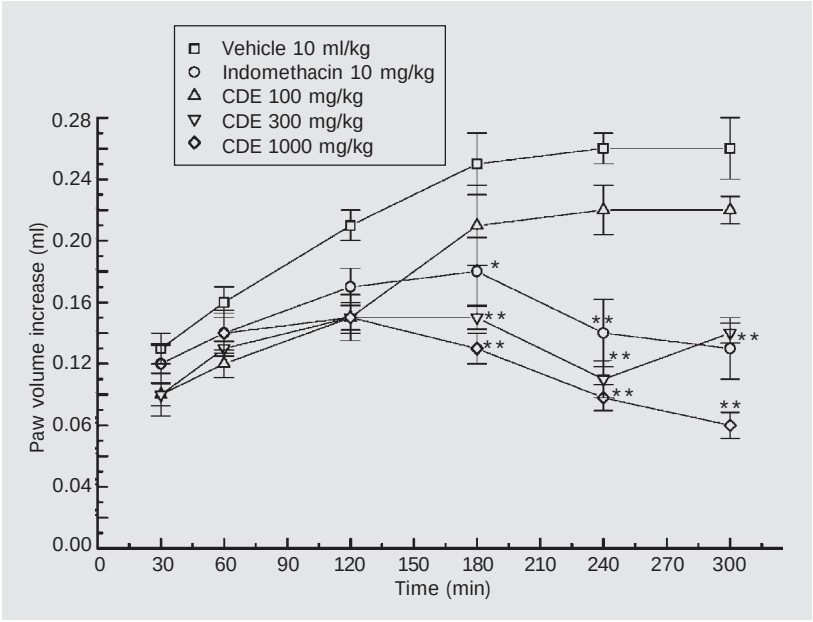

Figure 1. Effect of the crude dichloromethane extract (CDE) of *Cordia curassavica* (*po*) on carrageenin-induced paw edema in mice. Data are reported as means ± SEM for 6 mice in each group. *P<0.01 and **P<0.001 compared to vehicle (Duncan test).

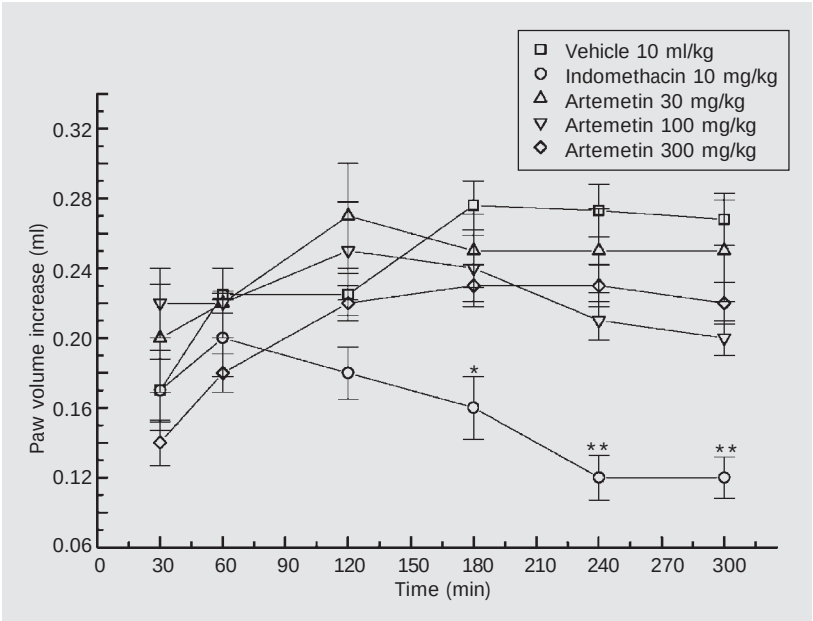

Figure 2. Effect of the artemetin fraction from *Cordia curassavica* (*po*) on carrageenin-induced paw edema in mice. Data are reported as means ± SEM for 6 mice in each group. *P<0.01 and **P<0.001 compared to vehicle (Duncan test).

The data obtained in the present study indicate the presence of liposoluble substances that probably justify the folk use of extracts of this species. The artemetin-enriched fraction, when orally administered, did not produce antiedematogenic activity, suggesting that the activity of *C. curassavica* cannot be attributed only to the oral administration of this flavonoid. Other substances are currently being isolated from the dichloromethane extract to permit the identification of the active compounds.

The dichloromethane extract was selected for antinociceptive and phytochemical studies. The antinociceptive activity of this extract was evaluated by the inhibition of acetic acid-induced writhing in mice according to the method of Siegmund et al. (11), modified by Koster et al. (12). Six mice were used in each group. The dichloromethane extract (100, 300 and 1000 mg/kg, *po*) was administered 1 h before acetic acid injection. The dose of 100 mg/kg did not reduce the number of writhings, whereas 300 mg/kg reduced the number of writhings from 37.1 ± 2.28 (control) to 17.3 ± 1.34, and 1000 mg/kg reduced the number of writhings to 13.2 ± 1.44. Indomethacin (10 mg/kg, *po*), a reference substance, reduced the number of writhings to 12.2 ± 1.27. Nevertheless, in the hot-plate test (13), 100 to 1000 mg/kg of the dichloromethane extract had no activity, suggesting that its analgesic effect is restricted to inhibition of the inflammatory process.

References

1. Ficarra R, Ficarra P & Tommasni S (1995). Leaf extracts of some *Cordia* species analgesic and anti-inflammatory activities as well as their chromatographic analysis. *Fármaco*, 50: 245-256.
2. Angely J (1970). *Flora Analítica e Fitogeográfica do Estado de São Paulo*. Vol. 4. Editora Phyton, São Paulo, SP, Brazil.
3. Sertié JAA, Basile AC, Panizza S, Matida AK & Zelnik R (1990). Anti-inflammatory activity and sub-acute toxicity of artemetin. *Planta Médica*, 56: 36-40.
4. Sertié JAA, Basile AC, Panizza S, Matida AK & Zelnik R (1988). Pharmacological assay of *Cordia verbenacea*. Part 1. Anti-inflammatory activity and toxicity of the crude extract of the leaves. *Planta Médica*, 54: 7-10.
5. Shilin Y, Roberts MF & Philipson JD (1989). Methoxylated flavones and coumarins from *Artemisia annua*. *Phytochemistry*, 28: 1509-1511.
6. Winter CA, Risley EA & Nuss GW (1962). Carrageenan-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine*, 111: 544-547.
7. Alonso JR (1998). *Tratado de Fitomedicina*. 1st edn. ISIS Edicione S.R.S., Buenos Aires, Argentina.
8. Di Carlo G, Mascolo N, Izzo AA & Capasso F (1999). Flavonoids: old new aspects of a class of natural therapeutic drugs. *Life Sciences*, 65: 337-353.
9. Middleton EJ, Kandaswami C & Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: implication for inflammation, heart disease and cancer. *Pharmacological Reviews*, 52: 673-751.
10. Basile AC, Sertie JAA, Oshiro T, Caly KDV & Panizza S (1989). Topical anti-inflammatory activity and toxicity of *Cordia verbenaceae*. *Fitoterapia*, I.X: 260-263.
11. Siegmund EA, Cadmus RA & Lu G (1957). Screening of analgesics including aspirin type compound based upon the antagonism of chemically induced writhing in mice. *Journal of Pharmacology and Experimental Therapeutics*, 119: 421-425.
12. Koster R, Anderson N & Debber EJ (1959). Acetic acid for analgesics screening. *Federation Proceedings*, 18: 412.
13. Lapa AJ, Souccar C, Lima-Landman MTR & Lima TCM (2001). *Métodos de Avaliação da Atividade Farmacológica de Plantas Medicinais*. CYTED/CNPq, São Paulo, SP, Brazil.