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PHARMACOLOGICAL SCREENING OF *AGERATUM CONYZOIDES* L. (MENTRASTO)

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The pharmacological activities of a water extract (WE) of Ageratum conyzoides *L, a plant popularly known for its analgesic and anti-inflammatory properties, were studied* in vivo *and* in vitro *preparations. Oral administration (p.o.) of the water extract (WE, 0.1 to 5 g/kg) to rats and mice induced quietness and reduced the spontaneous motility. The sleeping time induced by sodium pentobarbital (50 mg/kg, i.p.) in mice was not altered by previous treatment with WE (2 g/kg, p.o.). The same treatment did not influence the paw edema induced by carrageenan or dextran, nor did it reduce the chronic paw edema induced by complete Freund's adjuvant or formaldehyde in rats. The tail flick response in immersion test and writhings induced by 0.8% acetic acid in mice were not altered by WE either. In isolated guinea-pig ilea WE (0.4 to 4 mg/ml) did not alter the EC50 values of histamine or acetylcholine, but reduced the maximal response to the agonists by 20 to 50%. WE (0.01 to 10 mg/ml) produced tonic contractions of the ileal smooth muscle proportional to the doses, reaching a maximum of 75% relatively to the maximum obtained with histamine. Those contractions were blocked by diphenhydramine (10 nM) and reduced by 32% in presence of atropine (10 nM). The results indicated that oral treatment of rodents with* A. conyzoides *L neither reduced the inflammatory edema nor did it decrease the reaction to pain stimuli.* In vitro *the extract presented an unexpected histamine-like activity characteristic of a partial agonist. The results did not confirm the popular medicinal indications of the plant.*

Key words: *Ageratum conyzoides* L – anti-inflammatory – analgesia – medicinal plant

Ageratum conyzoides L., family Compositae, is a medicinal plant known in Brazil as "mentrasto", "caatinga de bode", "erva de São João" among others. Medicinal teas of *A. conyzoides* L are currently used in Brazilian folk medicine as anti-inflammatory, analgesic and anti-diarrheic (Pio Correa, 1926).

The present experiments were designed to investigate the pharmacological activities of the water extract of *A. conyzoides* L. particularly those related to its reputed analgesic and anti-inflammatory properties.

MATERIAL AND METHODS

The dried leaves of *A. conyzoides* were extracted with 2% hot water for 30 min, concentrated and freeze-dried. Pharmacological tests were done using rats (200-300 g), mice (30-40 g) and guinea-pigs (300-400 g) of both sexes. Composition of the nutritive solution was, in mM: NaCl 135; KCl 5; $MgCl_2$ 1; $CaCl_2$ 1.8; $NaHCO_3$ 15; NaH_2PO_4 1; and glucose 11.1.

Mice and rats were treated orally (p.o.) or intraperitoneally (i.p.) with 0.1 to 5 g/kg of the plant water extract (WE) for observations of the general pharmacological effects induced *in vitro* as described by Malone (1977). Central nervous system depressant activity was investigated in mice by measuring the sleeping time induced by sodium pentobarbital (50 mg/kg, i.p.) after 30 min gavage of WE (1 and 2 g/kg) or saline (control) (Carlini & Burgos, 1979). Analgesic activity was evaluated in mice by determining the pain reaction time to immersion of the tail in a hot water bath (55 ^{o}C), and by counting the number of writhes induced by 0.8% acetic acid (0.1 ml/10 g, i.p.) (Koster et al., 1959). The anti-inflammatory activity was tested on the acute rat paw edema induced

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by either 1% carrageenan or 1% dextran. The contralateral hind-paw was injected with 0.1 ml saline for control. Paw volumes were measured hourly for 2 to 4 h using a pletysmograph. Chronic paw edema was induced by subcutaneous (s.c.) injection into the rat hind-paw of 0.1 ml Freund's adjuvant (Newbould, 1963) or formaldehyde (Selye, 1949). The paw swelling was measured daily during 10 successive days. The results were expressed as percentage of paw swelling induced by the flogistic agent relatively to the contralateral paw (Winter et al., 1962).

Isolated guinea-pig ilea preparations were used for *in vitro* studies and cumulative concentration-response curves were constructed to Acetylcholine (ACh, 10^{-10} – 10^{-4} M) and Histamine (His, 10^{-10} – 10^{-4} M) (van Rossum 1963), in the absence and presence of WE (0.4 to 4 mg/ml). Cumulative dose-response curves of WE (0.01-10 mg/ml) were obtained in the absence and presence of Atropine, or Diphenhydramine (10^{-8} M).

Drugs used were: Acetylcholine chloride, Atropine sulfate, Dextran, Histamine diphosphate, Indomethacin (Sigma); Carrageenan (Cialgas), Diphenhydramine hydrochloride (Aldrich), Sodium pentobarbital (Abott). *A. conyzoides* was provided by Dr J. F. A. Mattos, UFCE, Fortaleza – Ceará.

Results were expressed as means ± s.e. mean and differences among control and treated groups were determined using the Student's "t" test, at a significance level of $P < 0.05$.

RESULTS

Administration of 0.5 to 5 g/kg WE to mice and rats p.o. or i.p., induced ptosis, dyspnoea and decreased motor spontaneous activity. The effects were proportional to the dosis and more intense after intraperitoneal injection of the extract in both animal species.

Previous administration of WE (1 and 2 g/kg, p.o.) to mice did not alter the sleeping time induced by barbiturates compared to saline treated animals (131.4 ± 13.8 min, n = 10).

The basal tail flick latency in control mice was 2.4 ± 1.8 sec (n = 10). Those values did not vary significantly during 3 h determinations, and were not altered after administration of WE (2 g/kg). Intraperitoneal injections of 0.8% acetic acid to control mice induced 27 ± 5 writhes/30 min (cumulative counts); those responses were not altered in animals pretreated with WE (2 g/kg, p.o.).

Injection of either 1% carrageenan or 1% dextran into the rat foot pad induced an intense and acute inflammatory paw swelling which increased progressively reaching a maximum within 3 (30.0 ± 4.2%, n = 5) or 1 (59.8 ± 3.5%, n = 5) hours, respectively. Treatment of rats with WE (1 and 2 g/kg, p.o.) did not influence the paw edema induced by either carrageenan or dextran. In simultaneous control experiments using rats treated with indomethacin (10 mg/kg, p.o.) or diphenhydramine (60 mg/kg, p.o.) the maximal paw edema was inhibited by respectively, 55% and 81% of control (Fig. 1).

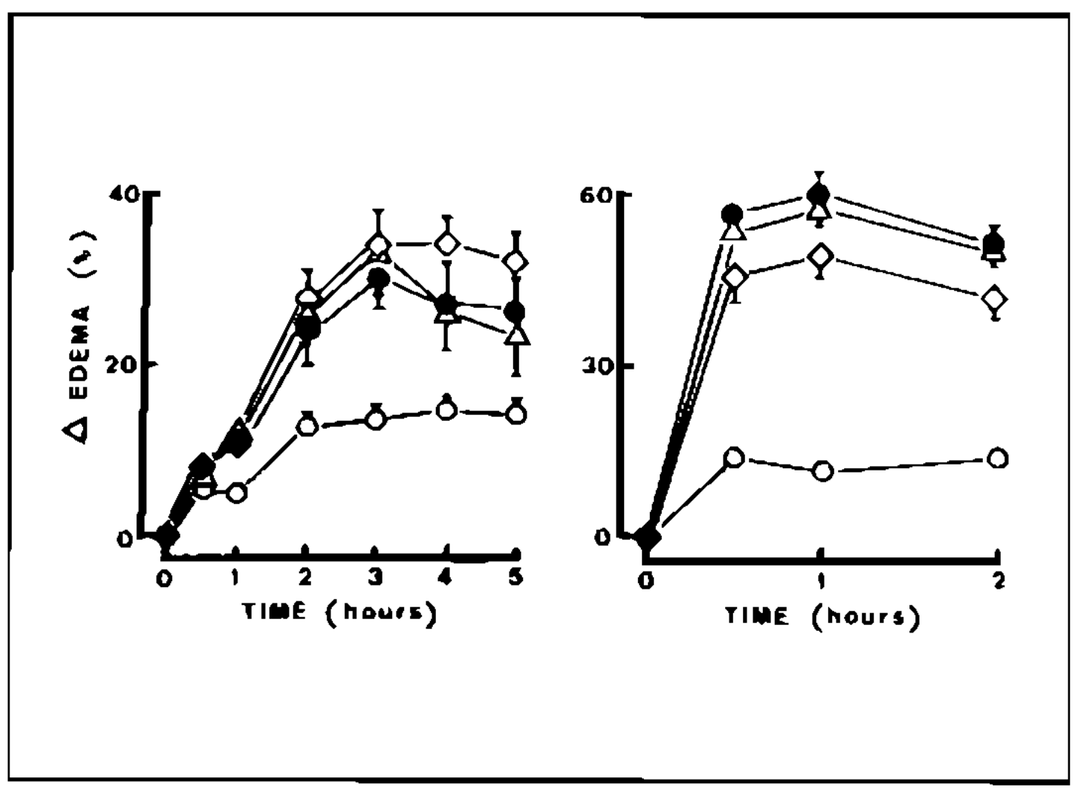

Fig. 1: hind-paw edema induced by carrageenan (left) or dextran (right) in control rats (●) and in animals pretreated orally with the extract of *Ageratum conyzoides* (1 g/kg – △; 2 g/kg – ◇) Indomethacin (10 mg/kg – ○, left) or Diphenhydramine (60 mg/kg – ○, right). Symbols and vertical bars are means ± s.e. mean of 5 animals each. Only values obtained in animals treated with Indomethacin and Diphenhydramine differed from control ($P < 0.05$).

Chronic injection of 0.1 ml complete Freund's adjuvant in the rat hind-paw caused a progressive edema which was maximal (70.4 ± 6.8%, n = 5) after 3 days. The rat paw edema chronically induced by 4% formaldehyde reached maximal values (35.0 ± 2.4%, n = 5) after 4 days. Daily administration of WE (1 and 2 g/kg, p.o.) did not influence either type of chronically induced rat paw edema. In both cases however, the paw swelling was reduced by 37% in animals pretreated with acetylsalicylic acid (200 mg/kg, p.o.).

Incubation of WE (1 and 4 mg/ml) in guinea-pig ileal preparations reduced the maximal contraction induced by ACh by 23% and 54%, without shifting the dose response curves. In other preparations, lower doses of WE (0.4 and 0.6 mg/ml) reduced the maximal contraction induced by His by 18% and 48%, respectively. Successive addition of progressive doses of the WE to the isolated guinea-pig ileum contracted the smooth musculature proportionally to the dose. Maximal contractions induced by the plant extract was 35% and 69% of those produced by ACh and His, respectively. Atropine (10^{-8} M) or diphenhydramine (10^{-8} M) reduced the contractile effects of WE by respectively, 32% and 79% without shifting the dose response curves (Fig. 2).

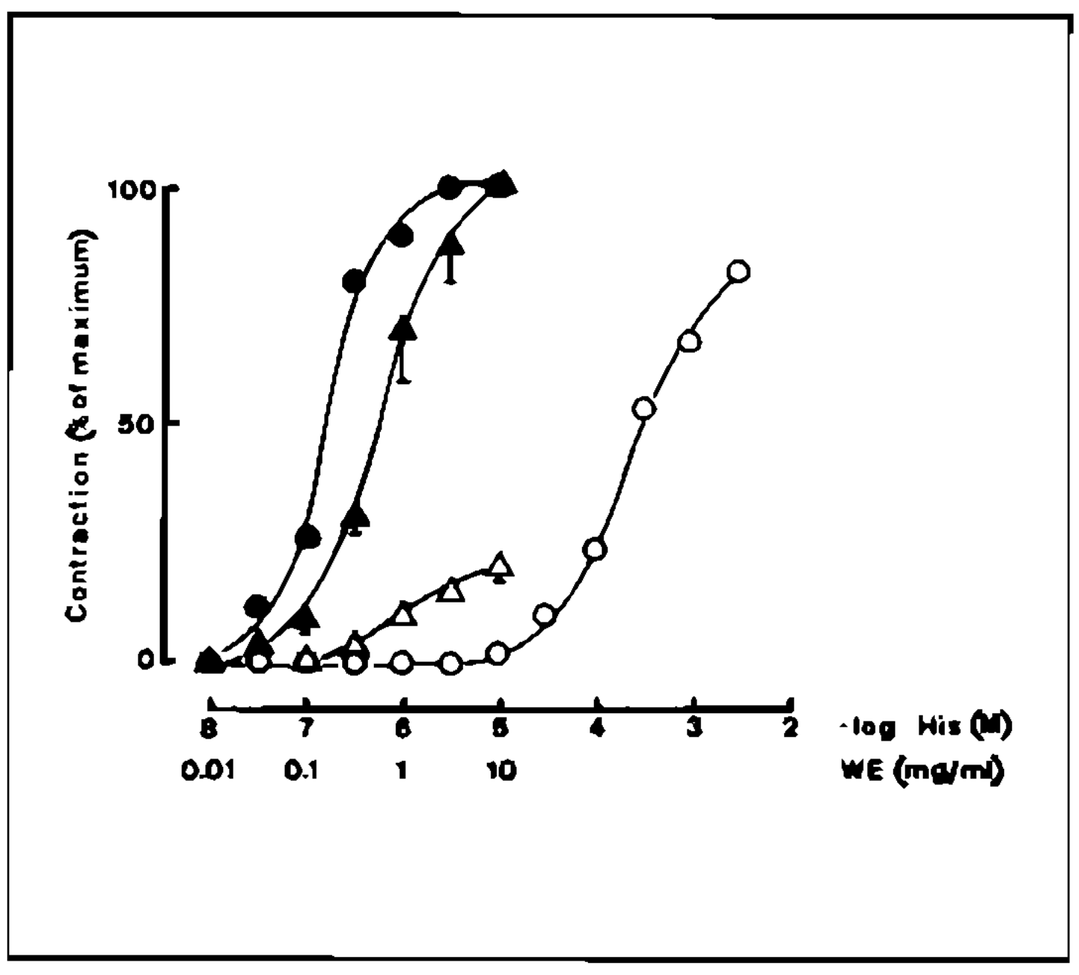

Fig. 2: cumulative dose-response curves constructed to Histamine (His) and the extract of *Ageratum conyzoides* L (WE) in isolated guinea-pig ilea, in the absence (His – ●; WE – ▲) and presence of 10^{-8} M Diphenhydramine (His – ○; WE – △). The maximal contraction induced by WE was 69% of that induced by His. Symbols and vertical bars are means ± s.e. mean of 4 experiments.

DISCUSSION

This study investigated the pharmacological activities of a water extract of *A. conyzoides* L (mentrasto), a plant reputed in folk medicine for its analgesic and anti-inflammatory properties.

Analgesic activity of the plant extract was not detected in our study as indicated by the data obtained on the tail flick response and on writhing induced by acetic acid in mice.

At the studied range of doses, the plant extract was also ineffective in inhibiting the acute as well as, the chronically induced paw edema in rats. Those results confirm previous studies done with extracts of *A. conyzoides* L. (Thomas et al., 1988). On the other hand, analgesic and anti-inflammatory effects of the plant water extract have been recently reported in clinical studies (Marques-Neto et al., 1988).

In vitro the extract induced tonic contractions of the guinea-pig ileum musculature, which were inhibited by atropine and diphenhydramine, suggesting a histamine-like activity. This action however, remains to be confirmed after purification of the plant extract.

In conclusion the presented data do not support the popular indication on the analgesic and anti-inflammatory activities of *A. conyzoides* L, and indicate the presence of a histamine-like constituent(s) in the plant extract.

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