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Assessment of antinociceptive and anti-inflammatory activities of *Porophyllum ruderale* aqueous extract

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Article

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Abstract: The present work investigated the antinociceptive and anti-inflammatory activities of the *Porophyllum ruderale* (Jacq.) Cass., Asteraceae, aqueous extract (PRAE). For this purpose, acetic acid writhing, paw licking induced by formalin, hot-plate and pleurisy tests were performed. The doses of 100, 200 and 400 mg/kg (*p.o.*) significantly inhibited the writhing 63.4, 89.6 and 94.8%, respectively, in comparison with control group. The lick of the paw 1st phase was reduced at the dose of 400 mg/kg (24.9%), while the 2nd phase had reduction at doses 200 and 400 mg/kg (23.1 and 34.4%), respectively. The PRAE inhibited the carrageenan-induced neutrophil migration to the peritoneal cavity in a higher dose ($p<0.05$). Taken together, our results suggest that the PRAE can constitute target potential for use in therapies of the pain and inflammation.

Introduction

Pain is a sensorial modality, which in many cases represents the only symptom for the diagnosis of several diseases, and often has a protective function. Throughout history man has used many different forms of therapy for the relief of pain, and medicinal herbs are highlighted due to their wide popular use (Melo et al., 2010). An example is *Papaver somniferum* L. (Papaveraceae), from which morphine has been isolated, and is regarded as the prototype of opiate analgesic drugs (Almeida et al., 2001).

Furthermore, as most of the plants were first used by the so-called primitive cultures, their occasional use by the White occidental culture was relegated to a second plan, being considered as sorcerer's therapeutics. Until recently, very little attention was given by the scientific community to the benefits, as accepted by folk medicine and the medicinal properties of the natural product (Barbosa-Filho et al., 2006a; Quintans-Júnior et al., 2008). In addition, nature is a rich source of biological and chemical diversity. The unique and complex structures of natural products cannot be obtained easily by chemical synthesis. A number of plants in the world have been used in traditional medicine remedies (Barbosa-Filho et al., 2006b).

Porophyllum ruderale (Jacq.) Cass., Asteraceae, is a ruderal aromatic herb known as "couve-cravinho". It is used in the folk medicine for cicatrisation, as anti inflammatory, fungicide, antibacterial, anti stress, to combat arterial hypertension, leishmaniosis, traumatism, antidote against snake poison, pain relief and rheumatism. Cicatrizing activity has been related with concentration of tannin, a type of phenolic compound (Lorenzi &Mattos, 2002). The aim of the present study was to evaluate the antinociceptive and anti-inflammatory properties of Porophyllum ruderale aqueous extracts (PRAE) from aerial parts in mice.

Material and Methods

Plant material

The aerial parts of *Porophyllum ruderale* (Jacq.) Cass., Asteraceae, were collected from the Areia Branca, Sergipe State, Brazil, in January 2008 and was identified by Ana Paula Prata from Federal University of Sergipe (DBI/UFS). A voucher specimen (nº 12.115) is deposited at the Herbarium of the Federal University of Sergipe.

Preparation of aqueous extract

An aqueous extract was obtained from the aerial parts of the *P. ruderale*. After harvesting, the aerial parts of the *P. ruderale* were adequately selected and dried in sterilizer with circulation and hot air renewal (Model MA-037) at 37 ºC until complete dehydration. Then, the aerial parts were weighed and triturated in electric mill to obtain a dust of fine granulation. The preparation of the PRAE was done by adding 2000 mL of distilled water to 400 g of aerial parts and kept at 77 ºC for 30 min. Finally, PRAE was filtered in vacuum, lyophilized and stored in the dark at -12 ºC. In the moment of the use the extracts was dissolved in saline+1 drop of Tween-80 0.2% (vehicle) in the desired concentration.

Animals

Male Swiss mice (25-30 g) were kept in a controlled temperature room (21±2 °C), light–dark cycles of 12 h each, and were allowed free access to food (Purina chow) and water. The experiments were performed with the approval of the Committee for the Use of Animals in Experiments of the Universidade Federal de Sergipe (CEPA/UFS Nº 03/08).

Drugs

Dexamethasone, morphine and polyoxyethylene-sorbitan monolated (Tween 80) was purchased from Sigma (USA) and acetilsalicilic acid (Aspirin), was obtained from Neoquímica (Brazil). All drugs and the *P. ruderale* aqueous extract (PRAE) were administered orally in volumes of 0.1 mL/10 g.

Acetic acid-induced writhing

This test was done using the method described by Koster et al. (1959) and Broadbear et al. (1994). Muscular contractions were induced by intraperitoneal injection (*i.p.*) of a 0.85% solution of acetic acid (10 mL/kg) to a group of six mice (n=6/group). The number of muscular contractions was counted for 15 min after injection and the data represents the average of the total number of writhes observed. PRAE was administered in doses of 100, 200 and 400 mg/kg (*p.o.*). The reference drug, aspirin, was dissolved in saline+1 drop of Tween-80 0.2% (vehicle) (300 mg/kg) and was administered to different groups of the mice 1 h before the acetic acid administration.

Formalin test

The method used was similar to that described previously by Hunskaar & Hole (1987). Twenty microlitres of 1% formalin was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw were taken as an indicator of pain response. Responses were measured for 5 min (first phase) and 15-30 min after formalin injection (second phase). PRAE (100, 200 and 400 mg/kg, *p.o.*) and aspirin (300 mg/kg, *p.o.*) were administered 60 min before formalin injection. Animals control received the same volume of saline solution orally.

Hot plate test

The hot-plate test was used to measure response latencies according to the method described by Eddy & Leimback (1953). Animals were placed on an Insight® hot-plate (Model EFF-361) maintained at 55±1 °C and the time between placement of the animal on the hot-plate and the occurrence of either the licking of the hind paws, shaking or jump off from the surface was recorded as response latency. Mice with baseline latencies of more than 10 s were eliminated from the study 24 h later. The cut-off time for the hot plate latencies was set at 30 s. Animals were treated with PRAE (100, 200 and 400 mg/kg, *p.o.*) 60 min before the experiments. Animals control received the same treatment to abdominal constriction test.

Leukocyte migration to the peritoneal cavity

The leukocyte migration was induced by injection of carrageenan (1%, *i.p.*, 0.25 mL) into the peritoneal cavity of mice 1 h after administration of *Porophyllum ruderale* (100, 200 and 400 mg/kg, *i.p.*, n=6), dexamethasone (2 mg/kg, *s.c.*, n=6) or vehicle (saline+1 drop of tween 80 0.2%, n=6) by modification of the technique previously described by Matos et al. (2003). The animals were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*) and were euthanized by cervical dislocation 4 h after carrageenan injection. Shortly after, saline containing EDTA (1 mM, *i.p.*, 3 mL) was injected. Immediately a brief massage was done for further fluid collection, which was centrifuged (5,000 rpm, 5 min) at room temperature. The supernatant was disposed and the precipitate was responded in saline. An aliquot of 10 µL from this suspension was dissolved in 200 µL of Turk solution and the total cells were counted in a Neubauer chamber, under optic microscopy. The results were expressed as the number of neutrophils/mL. The percentage of the leukocyte inhibition=(1-*T*/*C*) x 100, where T represents the treated groups leukocyte counts and *C* represents the control group leukocyte counts.

Statistical analysis

The obtained data was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett`s test. In all cases differences were considered significant if $p<0.05$. The percent of inhibition by an antinociceptive agent was determined for the acetic acid-induced writhing and formalin tests using the following formula (Reanmongkol et al., 1994): Inhibition %=100. (control-experiment)/control.

Results

Acetic acid-induced writhing

Table 1 shows that PRAE significantly ($p<0.001$) reduced, in a dose-dependent manner, the number of writhing movements induced of the acetic acid solution. PRAE treatment produced a similar effect to aspirin (300 mg/kg, *p.o.*), standard drug.

Table 1. Effect of PRAE or aspirin on writhing induced by acetic acid.

Treatment	Dose (mg/kg)	Number of writhings[a]	% Inhibition
Vehicle	-	29.0±1.9	-
PRAE	100	10.6±2.4[b]	63.4[c]
PRAE	200	3.0±1.5[b]	89.6[d]
PRAE	400	1.5±0.5[b]	94.8[d]
Aspirin	300	0.6±0.3[b]	97.9[d]

n=6; [a]Values represent mean±S.E.M.; [b]$p<0.001$ (one-way ANOVA and Dunnett`s test), significantly different from control; [c]$p<0.01$ (Fisher`s test), significantly different from control; [d]$p<0.001$ (Fisher`s test), significantly different from control.

Formalin test

As shown in Table 2, in the first phase, PRAE significantly did not reduce the time of licking compared with control group. However, PRAE significantly inhibited ($p<0.05$) the second phase of the formalin test.

Hot plate test

Table 3 shows the results of the hot plate test. All doses of PRAE were ineffective in inhibiting the time of reaction to the thermal stimulus compared to control (vehicle). Reference drug (morphine, 5 mg/kg, *i.p.*) significantly increased ($p<0.01$) the time of reaction.

Leukocyte migration to the peritoneal cavity

Figure 1 shows the inhibitory effect of PRAE on carrageenan-induced responses in higher dose ($p<0.05$) and dexamethasone (2 mg/kg, *s.c.*) significantly decreased of the leukocyte migration (predominantly neutrophils migration).

Discussion

Porophyllum ruderale is used in folk medicine for cicatrisation, as anti inflammatory pain relief and rheumatism. In contrast, there is little pharmacological information about the plant specie on literature. For the preliminary antinocipetive activity assessment of *P. ruderale* aqueous extracts (PRAE) were tested on acetic acid-induced writhing and formalin tests in rodents.

Table 2. Effect of PRAE or aspirin on formalin-induced pain.

Treatment	Dose (mg/kg)	Number of licks (s)			
		0-5 min		15-30 min	
		Score of pain[a]	% inhibition	Score of pain[a]	% inhibition
Vehicle	-	57.3±4.8	-	54.1±4.4	-
PRAE	100	49.3±7.2	13.9	51.5±3.7	4.8
PRAE	200	46.6±4.4	18.7	41.6±5.4[b]	23.1[d]
PRAE	400	43.0±6.9	24.9[d]	35.5±4.0[b]	34.4[d]
Aspirin	300	29.9±3.8[b]	47.8[d]	7.5±2.2[c]	86.1[e]

n=6; [a]Values represent mean±S.E.M.; [b]$p<0.05$ (one-way ANOVA and Dunnett`s test), significantly different from control; [c]$p<0.001$ (one-way ANOVA and Dunnett`s test), significantly different from control; [d]$p<0.05$ (Fisher`s test), significantly different from control; [e]$p<0.001$ (Fisher`s test), significantly different from control.

Table 3. Antinociceptive effect of PRAE on the hot plate test in mice

Treatment	Dose (mg/kg)	Reaction time (s)[a]				
		Basal	0.5h	1h	1.5h	2h
Vehicle	-	8.8±1.0	10.1±0.7	12.6±0.8	18.0±0.7	15.8±1.5
PRAE	100	10.4±1.2	14.6±2.5	15.0±2.0	15.1±2.5	18.5±3.0
PRAE	200	9.7±0.9	11.6±1.5	12.0±1.3	12.3±2.1	11.1±0.9
PRAE	400	11.5±1.0	13.3±1.1	14.0±1.2	18.0±3.4	18.0±2.9
Morphine	5	7.0±1.0	29.0±0.5[b]	29.8±0.1[b]	30.0±0.0[b]	29.8±0.1[b]

n=6; [a]Values represent mean±S.E.M.; [b]$p<0.001$ (one-way ANOVA and Dunnett`s test), significantly different from control.

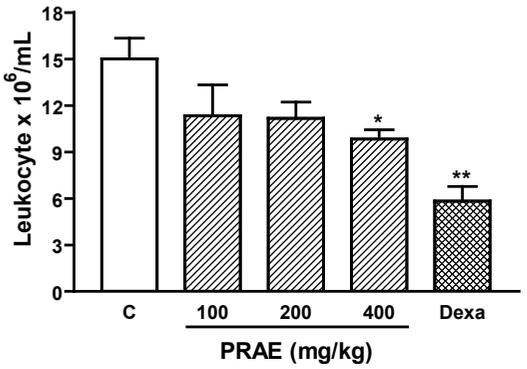

Figure 1. Effect of PRAE on leukocyte migration into the peritoneal cavity induced by carrageenan. Groups of mice were pre-treated with vehicle (C, 0.1 mL/10 g, *i.p.*, open column), dexamethasone (Dexa, 2 mg/kg, *s.c.*, cross-hatched column), or *P. ruderale* at doses of 100, 200, and 400 mg/kg (*i.p.*, right-hatched columns) 1 h before carrageenan (1%, 0.25 mL, *i.p.*)-induced peritonitis. Cell counts were performed at the time 4 h after the injection of carrageenan. Each value represents the mean±s.e.m. Asterisks denote statistical significance, *p<0.05 and **p<0.01 related to control group. ANOVA followed by Dunnett's test (n=6).

The inhibitory effect of PRAE in the writhing test is shown in Table 1. PRAE significantly inhibited the writhing in mice in a dose-dependent manner. Acetic acid-induced writhing is a standard, simple, and sensitive test for measuring analgesia induced by both opioids and peripherally acting analgesics (Hunskaar & Hole, 1987). This test, besides being the most appropriate antinociceptive model for opioids (Hayes et al., 1987), is also commonly employed as a visceral inflammatory pain model (Barber & Gottschlich, 1992). In acetic acid-induced abdominal writhing, pain is elicited by the injection of an irritant such as acetic acid into the peritoneal cavity which produces episodes of characteristic stretching (writhing) movements, and inhibition of the number of episodes by analgesics is easily quantifiable. Additionally, these results support the hypothesis of PRAE participation in the inhibition of prostaglandin synthesis, as the nociceptive mechanism involves the process or release of arachidonic acid metabolites via cyclooxygenase (COX) and prostaglandin biosynthesis (Duarte et al., 1988) during abdominal writhing induced by acetic acid.

Formalin test produced a distinct biphasic response and different analgesic drugs may act differently in the early and late phases of this test. Therefore, the test can be used to clarify the possible mechanisms of antinociceptive effect of a proposed analgesic (Tjolsen et al., 1992). Centrally acting drugs such as opioids inhibit both phases equally (Shibata et al., 1989) but, peripherally acting drugs, such as aspirin, indomethacin and dexamethasone only inhibit the late phase (Hunskaar & Hole, 1987; Rosland et al., 1990). The effect of PRAE on the second phases of formalin test suggests that its activity may be resulted from it's peripherally mechanism.

Based on the results of this study, we can suggest that the antinoceceptive effect of PRAE may be attributed to inhibition of prostaglandin release and other mediators involved in this test (Di Rosa et al., 1971; Melo et al., 2008). Moreover, the hot plate test checked a possible central antinociceptive effect of the PRAE since this is considered a specific test for central pain analysis. In this test, PRAE was not able to interfere with nociception.

Mild analgesics, as aspirin, lack antinociceptive action in thermally motivated tests such as hot plate test, but have significant antinociceptive activity in tonic tests (writhing and formalin tests), which are characterized by the direct chemical stimulation of nociceptors. Since, it has been reported that thermally motivated and tonic tests elicit the selective stimulation of A-γ fibers and C fibers, respectively (Yeomans et al.,1996), it is tempting to propose that PRAE may interfere with the transmission of both fibers or a common pathway.

In other set of experiments, the anti-inflammatory effect of PRAE was evaluated through carrageenan-induced peritonitis. Cell recruitment during inflammation depends on the orchestrated release of local mediators which are responsible for local vascular and tissue changes as well as for the recruitment of host defense cells (Luster et al., 2005). The inflammation induced by carrageenan involves cell migration, plasma exsudation and production of mediators, such as nitric oxide, prostaglandin E_2, IL-1β, IL-6 and TNF-α (Salvemini et al., 1996; Loram et al., 2007). These mediators are able to recruit leukocytes, such as neutrophils, in several experimental models. The PRAE inhibited leukocyte migration induced by i.p. injection of carrageenan (in peritonitis model). A putative mechanism associated with this activity may be inhibition of the synthesis of many inflammatory mediators whose involvement in the cell migration is well-established.

These experiments demonstrated some of the pharmacological properties of PRAE. Its analgesic and anti-inflammatory actions have been compared with substances that are considered standards for such activities. Additionally, our results suggest that PRAE has consistently shown to act peripherally on inflammatory mediators especially prostaglandins. The blockade of phase 2 of formalin test was typical of substances that antagonize cyclooxygenase, an enzyme which produces prostaglandins responsible for the genesis of fever and inflammation. Further studies are necessary to elucidate the mechanism behind its traditional effects.

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