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ORIGINAL ARTICLE

Antinociceptive and anti-inflammatory effects of *Costus spicatus* in experimental animals

Lucindo J. Quintans Júnior[1], Marília T. Santana[1], Mônica S. Melo[1], Damião P. de Sousa[1], Ivandilson S. Santos[1], Rosana S. Siqueira[1], Tamires C. Lima[1], Gabriela O. Silveira[1], Ângelo R. Antoniolli[1], Luciano A. A. Ribeiro[2], and Márcio R. V. Santos[1]

[1]*Departamento de Fisiologia, Universidade Federal de Sergipe (DFS/UFS), São Cristóvão, Sergipe, Brazil, and* [2]*Colegiado de Ciências Farmacêuticas, Núcleo de Estudos e Pesquisas de Plantas Medicinais, Universidade Federal do Vale do São Francisco (CFARM/NEPLAME/UNIVASF), Petrolina, Pernambuco, Brazil*

Abstract

Context: *Costus spicatus* Swartz (Costaceae), commonly called "cana-do-brejo'" in Brazil's northeast, is a medicinal plant found in wet coastal forests. In folk medicine an infusion of the aerial parts is taken to treat inflammation and pain.

Objective: The methanol extract obtained from the leaves of *Costus spicatus* (MECs) was evaluated for antinociceptive and anti-inflammatory activities.

Methods: Analgesic and anti-inflammatory activities were studied by measuring nociception through acetic acid, formalin, and hot-plate tests, while inflammation was induced by carrageenan. All experiments were conducted with experimental animals.

Results and discussion: Following oral administration, MECs (100, 200, and 400 mg/kg) significantly reduced the number of writhes (52.8, 43.1, and 55.3%, respectively) in the writhing test and the number of paw licks during phase 1 (61.9, 54.1, and 92.1%) and phase 2 (62.5, 82.9, and 98.1%, all doses) during the formalin test when compared to the control group animals. The reaction time during the hot-plate test was increased significantly and was dose-dependent, whereas pretreatment with naloxone rigorously reduced the analgesic potential of MECs, which suggested participation of the opioid system in the modulation of pain induced by MECs. Such results were unlikely to be provoked by motor abnormality, as MECs-treated mice did not exhibit any performance alteration during the Rota-rod test. The administration of 200 and 400 mg/ kg (i.p.) of MECs exhibited an anti-inflammatory effect during the carrageenan test, which was based on interference with inflammatory mediator synthesis.

Conclusion: We conclude that MECs has antinociceptive and anti-inflammatory activities in rodents.

Keywords: Costus spicatus*; inflammation; nociception; acetic acid; carrageenan; formalin; hot-plate*

Introduction

Medicinal plants, considered to possess therapeutic properties, have been used since the beginning of human civilization to treat different diseases, and the use of this effective strategy for the promotion of human health has significantly increased in recent years (Calixto et al., 2000). This fact is related to several factors, including the safety, effectiveness, and better quality control of phytomedicines available on the market today (De Souza et al., 2009). Although in recent years notable progress has been made concerning the development of natural therapies, there is an urgent need to discover effective and potent analgesic agents (Calixto et al., 2000; Barbosa-Filho et al., 2006; Melo et al., 2008).

Address for Correspondence: Lucindo J. Quintans Júnior, Departamento de Fisiologia, Universidade Federal de Sergipe – UFS, Av. Marechal Rondom, s/n, São Cristóvão, Sergipe, Brazil. Tel: +55-79-2105-6645. Fax: +55-79-3212-6640. E-mail: lucindo_jr@yahoo.com.br, lucindo@pq.cnpq.br

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Costus spicatus Swartz (Costaceae), popularly known as "cana-do-brejo" in Brazil's northeast, is a medicinal plant found in wet coastal forests. The rhizomes of this plant are used in folk medicine as a diuretic, a hypoglycemic, for the treatment of complaints of the bladder and urethra, and to expel kidney stones (Manfred, 1947; Gasparri, 2005). An infusion of the aerial parts of this plant is taken to treat colds, sore throats, dysentery, and diarrhea (Cruz, 1965). Flavonol glycosides have been isolated from the leaves and have demonstrated an inhibitory activity on nitric oxide production by activated macrophages (Da Silva et al., 2000). Additionally, the same group showed that polysaccharides isolated from the fresh stems of *C. spicatus* inhibited capillary permeability and demonstrated phagocytosis-stimulating properties in rodents (Da Silva & Parente, 2003). It has been reported that some flavonol glycosides have an anti-inflammatory effect through the inhibition of some macrophage functions involved in the inflammatory process, such as nitric oxide production (Fang et al., 2005). Other reports show that the plant extract, rich in flavonoids, has antinociceptive properties due to a possible interaction with the opioid system (Ghannadi et al., 2005; Maleki-Dizaji et al., 2007).

To date, no data exist about the possible antinociceptive and anti-inflammatory activities of *C. spicatus* in experimental animals. Thus, the purpose of the present study was to evaluate the antinociceptive and anti-inflammatory effects of the methanol extract obtained from the leaves of *C. spicatus* on rodents.

Materials and methods

Plant material

The leaves of *C. spicatus* were collected in November 2008 in São Cristóvão, Sergipe, Brazil. A voucher specimen (ASE 11453) is preserved in the Herbarium of the Departamento de Biologia at the Universidade Federal de Sergipe (UFS), Sergipe, Brazil.

Preparation of plant extract

The dried leaves of the plant (2.100 kg) were powdered and extracted exhaustively at room temperature with methanol in successive phases. After evaporation of the solvent under reduced pressure, 260 g of the methanol extract of *C. spicatus* (MECs) was obtained.

Drugs

Acetic acid, carrageenan, diazepam, and polyoxyethylene-sorbitan monolate (Tween 80) were purchased from Sigma (USA). Morphine (MOR), naloxone (NAL), indomethacin (INDO), and aspirin (acetylsalicylic acid) were purchased from União Química Farmacêutica Nacional (Brazil). The MECs was administered *per os* (p.o.) in volumes of 0.1 mL/10 g (mice) and 0.1 mL/100 g (rats), and the doses (100, 200, and 400 mg/kg) were adjusted for these respective volumes.

Animals

All experiments were performed on male Swiss mice (28–33 g) and male Wistar rats (180–200 g), housed in numbers of 20 or five to each cage, respectively, under a 12 h light/12 h dark cycle (lights on at 6:00 h) in a temperature controlled room (22 ± 2°C) with free access to laboratory chow and tap water. Mice were acclimatized to the laboratory conditions for at least 1 h before tests, which were carried out between 9:00 and 17:00 h. All experiments were conducted under the ethical guidelines of the International Association for the Study of Pain (IASP) (Zimmermann, 1983). Besides this, all experimental procedures were previously approved by the Committee on the Ethical Use of Animals at the Federal University of Sergipe (CEPA/UFS # 58/07), where the study was conducted. Animals were used only once throughout the experiments. The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of drug treatments.

Acetic acid-induced writhing

The acetic acid test was done using the method described by Koster et al. (1959) and modified by Broadbear et al. (1994). Initially, the mice were divided into five groups ($n = 8$). Subsequently, MECs (100, 200, and 400 mg/kg), vehicle (saline/Tween 80 0.2%, as control group), and aspirin (200 mg/kg) were administered p.o. 60 min before an injection of 0.25 mL per animal of acetic acid (0.85%). Each animal was isolated in an individual observation chamber, and 15 min after acetic acid injection the cumulative number of writhing responses was recorded for 15 min.

Formalin-induced pain

The formalin test was carried out as described by Hunskaar and Hole (1987). The animals were divided into six groups ($n = 8$) and treated with vehicle (control, p.o.), MECs (100, 200, and 400 mg/kg, p.o.), MOR (5 mg/ kg, s.c.), or aspirin (200 mg/kg; p.o.). After 60 min, 20 μL of a 2.5% formalin solution (0.92% formaldehyde) in a phosphate buffer (pH 7.2) was injected into the dorsal surface of the left hindpaw using a microsyringe with a 26-gauge needle. The duration of paw licking was measured from 0 to 5 min (first phase) and 15 to 30 min (second phase) after formalin administration.

Hot-plate test

The hot-plate test was carried out as described by Eddy and Leimbach (1953). In this test, the reaction of mice to painful stimulus was measured. Mice were placed individually on a metal plate heated to 52 ± 0.5°C and covered with a glass cylinder (25 cm high, 15 cm in diameter). The time (in seconds) elapsing to the first pain response (licking of the forepaws or jumping) was determined by a stop-watch and then recorded. The experiments were conducted 60 min following the p.o. administration of MECs (100, 200, and 400 mg/kg). The effect of pretreatment with NAL (1.5 mg/kg, i.p.) on the antinociception produced by MECs (400 mg/kg) and morphine (5 mg/kg, s.c.) was determined.

Rota-rod test

Initially, mice able to remain on the Rota-rod apparatus (AVS®, Brazil) longer than 180 s (at 9 rpm) were selected 24 h before the test (Rosland et al., 1990). Then, the selected animals were divided into five groups ($n = 8$) and treated with vehicle (control, p.o.), MECs (100, 200, and 400 mg/kg, p.o.), and diazepam (3 mg/kg, i.p.). Sixty minutes later, each animal was tested on the Rota-rod and the time (in seconds) that they remained on the bar, for up to 180 s, was recorded 60 min after treatment.

Carrageenan-induced edema test

Acute hindpaw edema was produced by injecting 0.1 mL of carrageenan (1%, prepared as a suspension in distilled water plus Tween 80 at 0.2%) locally into the subplantar aponeurosis of the right hindpaw of rats (Winter et al., 1962). Animals were divided into five groups, six rats per group. MECs was administered p.o. at different doses (100, 200, and 400 mg/kg) in the absence and presence of the standard drug indomethacin (INDO, 10 mg/kg, p.o.), and vehicle (p.o.) was given to a control group. MECs and INDO were administered 1 h prior to injection of carrageenan (Amresh et al., 2007). Paw volume was measured by dislocation of the water column of a plethysmograph (LE 7500; PanLab, Spain) immediately after carrageenan application (time zero) and at 1, 2, 3, and 4 h after its administration.

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM) and the differences between control and treated groups were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's or Fisher's test. In all cases differences were considered significant if $p < 0.05$.

The percentage 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. The percentage of inhibition of edema volume between treated and control groups was calculated using the following formula: inhibition % = $100 \times (V_c - V_t)/V_c$, where V_c and V_t represent the mean increases in paw volume in the control and treated groups, respectively.

Results

In control group mice, the number of writhes during the 15 min test period was 12.3 ± 1.5 ($n = 8$). The treatment of the animals with MECs (100, 200, and 400 mg/kg, p.o.) produced a significant and dose-dependent inhibition of the number of writhes when compared to the control (Table 1). The inhibition induced by 400 mg/kg MECs was similar to that produced by 200 mg/kg aspirin (55.3 and 68.3%, respectively).

In this work, MECs demonstrated analgesic effects on both the first (0–5 min) and second phases (15–30 min) of formalin-induced pain. These phases correspond to neurogenic and inflammatory pain, respectively. Neurogenic-induced pain was blocked only at 400 mg/kg (92.1%, $p < 0.001$), whereas all doses of MECs significantly blocked inflammatory pain. Aspirin (200 mg/kg, p.o.) was significantly active (58.6%, $p < 0.001$) in the second phase, i.e. inflammatory pain (Table 2).

As demonstrated in Table 3, MECs administered at doses of 200 and 400 mg/kg caused a significant increase in hot-plate test response latency when compared to control group animals. Naloxone (1.5 mg/kg, i.p.) partially reversed the antinociceptive effect of the MECs.

In the Rota-rod test, MECs-treated mice did not show any significant motor performance alterations with doses of 100, 200, or 400 mg/kg (Figure 1). As might be expected, the central nervous system (CNS) depressant

Table 1. Effect of methanol extract of *C. spicatus* (MECs) or aspirin on writhing induced by acetic acid ($n = 8$).

Treatment	Dose (mg/kg)	Number of writhings[a]	% Inhibition
Vehicle	—	12.3 ± 1.5	—
MECs	100	5.8 ± 1.7[c]	52.8[d]
MECs	200	7.0 ± 0.8[b]	43.1[d]
MECs	400	5.5 ± 1.4[c]	55.3[d]
Aspirin	200	3.9 ± 1.9[c]	68.3[e]

[a]Values represent mean ± SEM.
[b]$p < 0.05$ (one-way ANOVA and Dunnett's test), significantly different from control.
[c]$p < 0.01$ (one-way ANOVA and Dunnett's test), significantly different from control.
[d]$p < 0.01$ (Fisher's test), significantly different from control.
[e]$p < 0.001$ (Fisher's test), significantly different from control.

diazepam (3 mg/kg) reduced the time of treated animals on the Rota-rod apparatus.

MECs presented a dose-dependent anti-inflammatory activity at all concentrations tested, with a significant difference at the concentration of 100 mg/kg, 4 h after carrageenan injection (Table 4).

Table 2. Effect of methanol extract of *C. spicatus* (MECs) or aspirin on formalin-induced pain ($n=8$).

		Number of licks (s)			
		0–5 min		15–30 min	
Treatment	Dose (mg/kg)	Score of pain[a]	% Inhibition	Score of pain[a]	% Inhibition
Vehicle	—	55.6 ± 5.4	—	51.4 ± 5.0	—
MECs	100	21.2 ± 6.5[c]	61.9[e]	19.3 ± 9.1[c]	62.5[e]
MECs	200	25.5 ± 6.7[b]	54.1[e]	8.8 ± 5.7[c]	82.9[e]
MECs	400	4.4 ± 1.7[c]	92.1[e]	1.0 ± 0.3[c]	98.1[e]
Aspirin	200	39.3 ± 14.7	29.3[d]	21.3 ± 12.8[c]	58.6[e]

[a]Values represent mean ± SEM.
[b]$p<0.01$ (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. Effect of methanol extract of *C. spicatus* (MECs) or morphine (MOR) on the hot-plate test in the absence and presence of naloxone (NAL) in mice ($n=8$).

	Dose	Reaction time (licking of the hindpaw) (s)[a]			
Treatment	(mg/kg)	Basal	0.5 h	1 h	1.5 h
Vehicle	—	7.5 ± 0.4	5.9 ± 0.7	5.5 ± 0.6	7.4 ± 1.3
MECs	100	5.3 ± 0.7	7.4 ± 1.6	5.7 ± 1.1	8.6 ± 1.2
MECs	200	7.3 ± 0.3	6.5 ± 1.0	15.1 ± 1.7[b]	7.9 ± 0.6
MECs	400	8.5 ± 1.0	17.2 ± 1.3[b]	17.1 ± 1.5[b]	16.9 ± 2.2[b]
MECs + NAL	400 + 1.5	6.4 ± 0.4	6.5 ± 0.6	7.8 ± 0.7	9.3 ± 1.1
MOR	5	7.1 ± 2.8	26.5 ± 4.5[c]	29.3 ± 3.5[c]	28.7 ± 4.1[c]
MOR + NAL	5 + 1.5	5.9 ± 4.1	9.4 ± 4.4	8.8 ± 2.0	9.5 ± 4.5

[a]Values represent mean ± SEM.
[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.

Discussion

In this study we evaluated the antinociceptive and anti-inflammatory effects of the methanol extract obtained from the leaves of *Costus spicatus* Swartz (MECs) using acetic acid-induced writhing, formalin, hot-plate, and carrageenan-induced edema tests in rodents.

MECs, at all doses, was able to inhibit acetic acid-induced writhing in mice; hence, it can be suggested that the analgesic effect of the extract is peripherally mediated (Melo et al., 2008). This method is not only simple and reliable, but also affords rapid evaluation of peripheral type analgesic action. 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 the inhibition of the number of episodes by analgesics is easily quantifiable

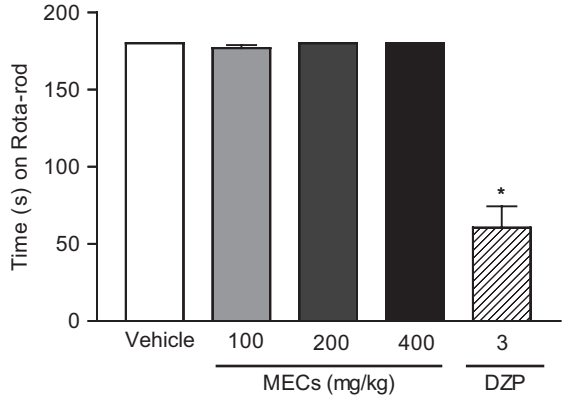

Figure 1. Time (s) on the Rota-rod apparatus observed in mice after p.o. treatment with vehicle (control), MECs (100, 200 and 400 mg/kg), or diazepam (DZP, 3 mg/kg, i.p.). The motor response was recorded for 180 s following drug treatment. Statistical differences vs. control group were calculated using ANOVA, followed by Dunnett's test ($n=8$). *$p<0.01$.

Table 4. Effect of methanol extract of *C. spicatus* (MECs) or indomethacin (INDO) on carrageenan-induced hindpaw edema in rats ($n=6$).

		Paw edema (mL)				
Treatment	Dose (mg/kg)	1	2	3	4	% Edema inhibition
Vehicle	—	0.35 ± 0.05	0.47 ± 0.04	0.57 ± 0.03	0.40 ± 0.03	—
MECs	100	0.18 ± 0.02[c]	0.27 ± 0.05[b]	0.48 ± 0.05	0.35 ± 0.04	28.5[d]
MECs	200	0.17 ± 0.03[c]	0.23 ± 0.05[b]	0.35 ± 0.03[b]	0.29 ± 0.06[b]	41.9[d]
MECs	400	0.12 ± 0.02[c]	0.19 ± 0.04[c]	0.29 ± 0.04[c]	0.29 ± 0.04[b]	50.3[e]
INDO	10	0.09 ± 0.03[c]	0.14 ± 0.03[c]	0.19 ± 0.06[c]	0.20 ± 0.03[c]	65.4[e]

[a]Values represent mean ± SEM.
[b]$p<0.01$ (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.

(Duarte et al., 1988). Furthermore, these results support the hypothesis of MECs participation in the inhibition of prostaglandin synthesis, since the nociceptive mechanism of abdominal writhing induced by acetic acid involves the process of release of arachidonic acid metabolites via COX (cyclooxygenase) and prostaglandin biosynthesis (Basbaum & Julius, 2001).

The results show that MECs, at the doses of 100, 200, and 400 mg/kg (p.o.), produced significant anti-nociception during the hot-plate and formalin tests. Substances and drugs that produce strong inhibitory effects in the hot-plate test can inhibit centrally induced pain and act as strong analgesics (Parkhouse & Pleuvry, 1979; Prado et al., 1990). This central analgesic action was confirmed by the blocking effect of naloxone (Belvisi et al., 1998). On the other hand, the formalin paw-licking test is a model of tonic pain and resembles human clinical pain conditions (Tjolsen et al., 1992). The observed activities of the extract in the hot-plate and formalin tests therefore suggest that MECs has strong analgesic activities. These findings support the use in folk medicine of the plant decoction for the treatment of headache.

Lack of motor coordination in the Rota-rod test is characteristic of drugs that reduce CNS activity, such as neuroleptics, anxiolytics, sedatives, and hypnotics (Sen & Chaudhuri, 1992). The animals treated with MECs did not present significant alterations in performance time on the Rota-rod apparatus, therefore not interfering with the motor coordination of the animals and/or discarding the muscular relaxant effect or even common neurotoxicity as some drugs with a depressant profile on the CNS.

The initial phase of carrageenan paw edema is mediated by histamine and serotonin, while the mediators in the later phase are suggested to be arachidonate metabolites (prostaglandins and leukotrienes) producing an edema dependent on the mobilization of neutrophils (Vinegar et al., 1987; Hwang et al.; 1996). In our experiments, the edematous response was significantly suppressed in rats pretreated with MECs in the first phase of edema, suggesting an inhibitory effect on the release of histamine and/or serotonin. MECs showed a significant inhibition of edema in the second and third phases, suggesting inhibition of 5-lipoxygenase and/or cyclooxygenase, both enzymes involved in the formation of prostaglandins and leukotrienes. This edematous response was also significantly reduced in rats pretreated with indomethacin, a compound known to be a cyclooxygenase inhibitor.

The ability of the MECs, in this study, to suppress abdominal writhes, increase the latency of reaction time in the hot-plate test, and inhibit both phases of formalin-induced pain, as well as suppress carrageenan-induced inflammation, confirm the analgesic and anti-inflammatory activities of MECs.

Conclusion

Taken together, all data presented in this work lead to the conclusion that the methanol extract obtained from the leaves of *C. spicatus* possesses analgesic and anti-inflammatory properties, which are probably mediated via the inhibition of prostaglandin synthesis as well as central inhibitory mechanisms. Therefore, the extract will be of potential benefit in the management of pain and inflammatory disorders.

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Declaration of interest

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