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Short communication

A comparative chemical and pharmacological study of standardized extracts and vanillic acid from wild and cultivated *Amburana cearensis* A.C. Smith

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ABSTRACT

The objectives of this work were to carry out a comparative chemical study and to evaluate the antinociceptive and anti-inflammatory activities of ethanol extracts (EtOHE) and vanilic acid (VA) from cultivated and wild *Amburana cearensis* A.C. Smith (Fabaceae), an endangered species used in Northeast Brazil for the treatment of asthma. The HPLC analysis of EtOHE, showed that coumarin (CM) and VA were the major constituents from the cultivated plant, while in the extract from the wild plant the major constituents were amburoside A (AMB) and CM. Pharmacological tests were performed with male Swiss mice or male Wistar rats acutely administered with 100–400 mg/kg, p.o. of EtOHEs or 12.5–50 mg/kg, p.o. of VA. EtOHEs from *A. cearensis* with 4, 7 or 9 months of cultivation significantly inhibited, from 32 to 64%, both phases of the formalin test in mice. Similar results were observed with the EtOHE from the wild species. VA significantly reduced both phases of the formalin test. This effect was partially reversed by naloxone. EtOHE from cultivated or wild *A. cearensis* inhibited the carrageenan (Cg)-induced mice paw edema. Furthermore, VA inhibited the paw edema and the leukocyte migration in rat peritoneal cavity induced by Cg. On the other hand, it did not inhibit the edema and the increase of vascular permeability induced by dextran in the rat paw. All together, these results indicate that the EtOHE from cultivated *A. cearensis* exhibit similar chemical and pharmacological profiles, as related to the wild plant. VA is, at least partially, responsible for these pharmacological effects. Its antinociceptive effect occurs by a mechanism partly dependent upon the opioid system, while the anti-inflammatory action was manifested in inflammatory processes dependent on polymorphonuclear cells and are probably related to the VA inhibition of cytokines as observed by others.

Introduction

In the state of Ceará, Brazil, *Amburana cearensis* A. C. Smith (Fabaceae), is popularly known as “cumaru”, “amburana” or “amburana-de-cheiro”. Its trunk bark and seeds are traditionally used as an antispasmodic, anti-inflammatory, antitussive, and mainly for the treatment of asthma (Correa 1984).

Several compounds have been isolated from the trunk bark of *A. cearensis*, including CM, protocatechuic acid, VA, flavonoids and phenol glucosides, amburosides A and B (Canuto and Silveira 2006; Bravo and Sauvain 1999). The toxicological study of the hydroalcoholic extract (HAE) from the wild trunk bark of *A. cearensis*, in rats did not show any toxic effects (Leal et al. 2003a). Furthermore, previous studies (Leal et al. 1997, 2000, 2003b, 2006, 2009) reported antinociceptive, anti-inflammatory, antioxidant and smooth muscle relaxant properties of its HAE and chemical constituents.

The large-scale use of wild *A. cearensis* as a medicinal plant, and in the formulation of the Syrup of Cumaru by Governmental Programs of Phytotherapy, in Northeast Brazil, and by the local pharmaceutical industry as well, are contributing to the decreased availability of the species, presently considered as an endangered one (Hilton-Taylor 2000). The goal of the present paper was to study, in a comparative way, chemical and pharmacological profiles of ethanolic extracts (EtOHE) from the wild and cultivated *A. cearensis*. The emphasis was on the anti-inflammatory activity as assessed by experimental models *in vivo*. Furthermore, the contribution of VA to the pharmacological effect of the cultivated plant was also studied.

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Table 1
Quantitative analysis by HPLC of the EtOHE from cultivated or wild *Amburana cearensis*.

Standard compounds	Cultivated plant				Wild adult plant Concentration g/100 g extract (RSD%[a])
	Retention time (min)	Concentration g/100 g extract (RSD%[a]) harvesting time (month)			
		4	7	9	
Protocatechuic acid	7.1	ND[b]	ND[b]	ND[b]	0.9 (2.5)
Vanillic acid	10.3	2.7 (13.4)	3.4 (4.3)	1.5 (6.1)	0.7 (6.5)
Coumarin	13.4	2.0 (7.0)	4.1 (6.5)	0.7 (1.8)	2.0 (1.5)
Amburoside A	14.2	ND*	ND*	0.4 (5.7)	5.3 (6.4)

All chromatography analyses were performed in triplicate.
[a] RSD: Relative standard deviation.
[b] ND: Not determined.

Materials and methods

Plant material

Trunk barks of wild adult *A. cearensis* were collected at the Quixeramobim region, Ceará State, Northeast Brazil. Voucher specimens (no. 837 and 847) were deposited at the Prisco Bezerra Herbarium, Federal University of Ceara. Seedlings obtained from *A. cearensis* seed germination were transplanted to garden beds (1.2 m × 10 m), containing 20 young plants each. Eight harvestings, starting at the 2nd month (60 days after sowing, DAS) until the 9th month (270 DAS), were performed monthly.

Plants extract, standards isolation and HPLC analysis

Every month, starting at the second month (60 DAS), 4 specimens (all plant) were harvested from the garden beds, dried at room temperature, ground and extracted by maceration for 24 h (4×) with ethanol. The ethanol extract from wild trunk barks of *A. cearensis* was obtained by Sohxlet extraction for 24 h, and the chemical constituents (VA, coumarin, protocatechuic acid and amburoside A) were isolated as described in a previous work (Canuto and Silveira 2006). EtOHE obtained from wild and cultivated *A. cearensis* were analyzed by HPLC according the method described previously (Canuto 2007).

Pharmacological experiments

Animals

Male Swiss mice (25–30 g) and Wistar rats (200–250 g) were housed in standard environmental conditions (23 ± 1 °C, humidity 60 ± 5%, and a 12 h/12 h dark/light cycle, light on at 7.00 a.m.). All experiments were conducted in accordance to the Guide for the Care and Use of Laboratory Animals, US Department of Health and Human Services, 1985.

Formalin test

Formalin-induced paw-licking was determined essentially as described by Hunskaar and Hole (1987). Swiss mice were treated with the EtOHE (100 and 200 mg/kg, p.o.) from the wild (trunk bark) or cultivated (all parts; 4, 7 and 9 months) plants, or with VA (25 and 50 mg/kg, p.o.), 60 min before the intraplantar formalin injection (1%, 30 μl). Control animals received vehicle (4% Tween 80 in water). In another set of experiments, mice administered with VA (50 mg/kg, p.o.) or morphine (5 mg/kg, s.c.) were also pretreated with naloxone (1 mg/kg, s.c.), before formalin.

Paw edema induced by carrageenan or dextran

The animals were pretreated with EtOHE (200 and 400 mg/kg, p.o.), VA (25 and 50 mg/kg, p.o.), indomethacin (5 mg/kg, p.o.), loratadine (5 mg/kg, p.o.) or vehicle (Tween 80, 4% in water), before receiving the injection of 50 μl of Cg, 1% (w/v) – mice) or dextran (1.5% v/w – rats) into the subplantar area of the right hind paw. The paw volume was determined with a pletismometer (Ugo Basile, Italy) (Levy 1969). The effect of test drug in the increase of the vascular permeability induced by dextran in rats was determined according to Gamsé et al. (1980).

Carragenan-induced neutrophil migration in rats peritoneal cavity

Wistar rats (150–200 g) were pretreated with VA (12.5 and 50 mg/kg, p.o.), dexamethasone (5 mg/kg, p.o.) or vehicle, and 1 h later they were injected with Cg (1%, i.p.). Total leucocyte counts in the wash fluid are expressed as number of cells/ml (Ferrándiz and Alcaraz 1991).

Statistical analysis

The results are presented as means ± S.E.M., and were analyzed by ANOVA, followed by Tukey as the *post hoc* test (GraphPad Prism program, USA). $p < 0.05$ was used as the significance level.

Results and discussion

Despite the existence of phytochemical, toxicological and pharmacological literature data (Leal et al. 1997, 2000, 2003a,b, 2006, 2008; Canuto and Silveira 2006) on the wild *A. cearensis*, the present work represents the first comparative study with standardized extracts from young cultivated and wild adult plants. The ^{1}H NMR spectra of all crude extracts from *A. cearensis* were compared, leading to the choice of extracts from 120 DAS (4 months), 210 DAS (7 months) and 270 DAS (9 months), based on a markedly different NMR profile. HPLC analyses of EtOHEs from wild and cultivated plants allowed us to detect and quantify in the species, four constituents (protocatechuic acid, VA, CM and AMB). The major constituents of cultivated plant were CM and VA, while in the EtOHE from wild plant were CM and AMB (Table 1).

The present work revealed that the acute treatment with EtOHE from cultivated (4, 7 or 9 months) or wild *A. cearensis* present antinociceptive and antiedematogenic effects in mice. The oral administration of EtOHE or VA inhibited both phases of the formalin response. In addition, the antinociceptive effects of VA as well as morphine were partially reversed by naloxone, indicating a partial involvement of the opioid system in this effect.

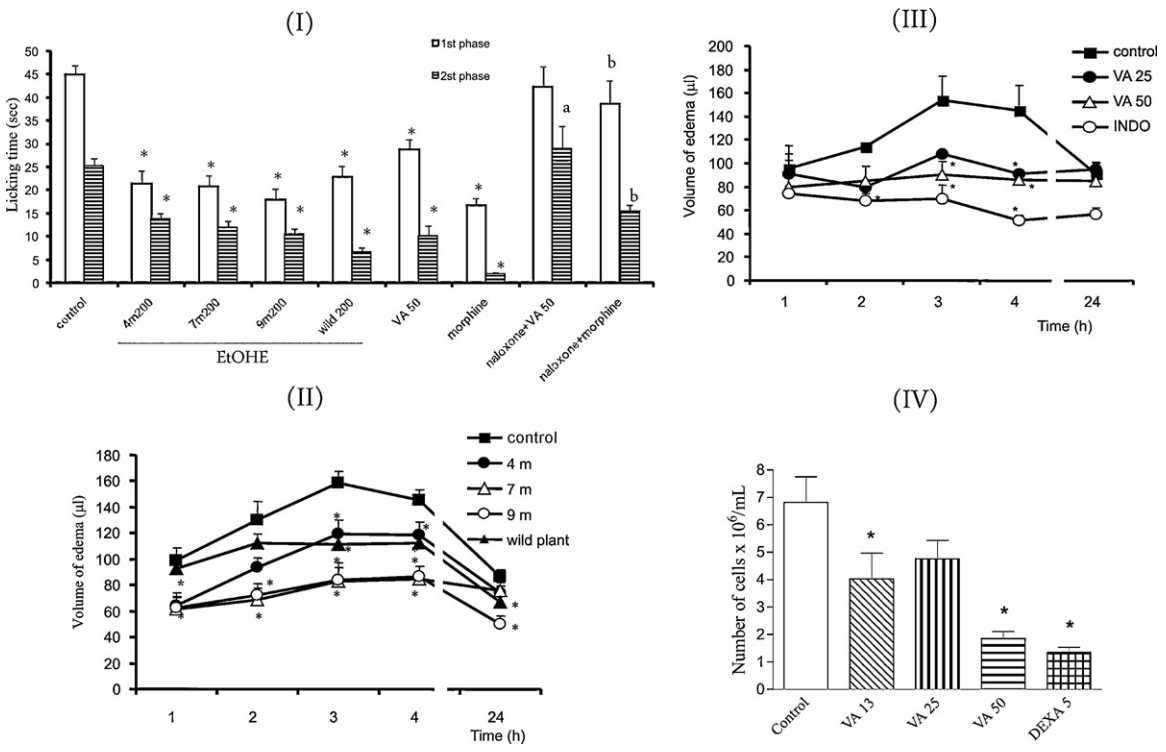

Fig. 1. Antinociceptive and anti-inflammatory effects of ethanolic extracts (EtOHE) and vanillic acid (VA) from *Amburana cearensis* in rodents. I: effects of EtOHE (cultivated, (4, 7 and 9 months (m) or wild plants: 200 mg/kg, p.o.), VA (50 mg/kg, p.o.) or morphine (MP, 5 mg/kg, s.c.) on the formalin-induced nociception in mice (6-–18 animals/group). II, III and IV: anti-inflammatory effects of EtOHE and VA on the carrageenan (Cg)-induced mice paw edema and Cg-induced rat peritonitis. In II and III, groups of 8-16 animals were treated with 400 mg/kg, p.o. of EtOHE (cultivated: 4, 7 and 9 m or wild plant), VA (25 and 50 mg/kg, p.o.), indomethacin (INDO, 5 mg/kg, p.o.) or vehicle (control), 60 min before the Cg injection (1%, 50 µL/paw). IV: Cg-induced rat peritonitis – the animals were treated with VA (13 and 50 mg/kg, p.o.), dexamethasone (DEXA, 5 mg/kg, p.o.) or vehicle (control), 60 min before the Cg injection (1%, 10 ml/kg). Results represent means ± S.E.M. * EtOHE, VA, MP, INDO, Dexa vs. control; [a] VA50 + naloxone vs. VA50; [b] MP5 + naloxone vs. MP5 ($p < 0.05$; ANOVA and Tukey as the *post hoc* test).

The initial formalin response was attributed to a direct algogenic effect of formalin on nociceptors, whereas the phase 2 was associated with the release of local endogenous mediators (serotonin, histamine, bradykinin and prostaglandins), responsible for sensitization of primary and spinal sensory neurons, and subsequent activation of nociceptors (Hunskaar and Hole 1987; Tjolsen et al. 1992). Our results suggest that the antinociceptive effects of EtOHE and VA from cultivated *A. cearensis* seem to be related mainly to their anti-inflammatory action. Confirming this hypothesis, the cultivated plant showed an antiedematogenic activity in paw edema induced by Cg in mice. Similarly to indomethacin, the standard drug, VA inhibited the paw edema induced by Cg in mice. On the other hand, the VA unlike loratadine did not interfere in the inflammatory process induced by dextran in rats.

Oral treatment of animals with VA at the highest dose (50 mg/kg) caused an inhibition in the accumulation of PMN in the peritoneal cavity of rats that was comparable to the effect of dexamethasone, standard drug. These results support the hypothesis that the anti-inflammatory action of VA from cultivated *A. cearensis* is possibly related to its ability to inhibit the accumulation of PMN in inflamed tissues (Fig. 1). Previous studies also showed the anti-inflammatory properties of phenolic acids, as well as of vanillic and protocatechuic acids (Fernandez et al. 1998; Chiang et al. 2003).

The present study shows that both cultivated and wild plants have similar phytochemical profiles. Furthermore, EtOHE from the cultivated plant, with 4, 7 or 9 months of cultivation, showed a similar pharmacological activity, as compared to the EtOHE from wild plant, although EtOHE from 4-month-species was somewhat less potent in paw edema models. VA is probably responsible, at least in part, for the pharmacological activities of the cultivated *A. cearensis* extracts, but the pharmacological importance of others chemical constituents from cultivated plant cannot be excluded. Furthermore, it has been recently observed (Itoh et al. 2009) that VA significantly decreased pro-inflammatory cytokine levels in a model of Con-A-induced liver injury in mice and this activity may also be involved with findings of the present study.

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