

Research article

Antinociceptive and antiedematogenic properties and acute toxicity of *Tabebuia avellanedae* Lor. ex Griseb. inner bark aqueous extract

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Abstract

Background: *Tabebuia avellanedae* is a tree from the *Bignoniaceae* family. Commonly known as "pau d'arco" in Brazil, its inner bark is used as analgesic, anti-inflammatory, antineoplastic and diuretic at the Brazilian northeast. A validation of the plant usage has not been previously performed.

Results: Antinociceptive and antiedematogenic effects of *Tabebuia avellanedae* Lor. ex Griseb. inner bark were measured by nociceptive experimental models in mice. A rat paw edema test induced by carrageenan (1%) was also performed in rats to assess the plant's antiedematogenic effect. The inner bark aqueous extract, administered *via* oral in three different concentrations, namely 100, 200 and 400 mg/Kg, reduced the nociception produced by acetic acid (0.6% in water, *i.p.*) by 49.9%, 63.7% and 43.8%, respectively. The aqueous extract (200 and 400 mg/Kg, *p.o.*) reduced formalin (1%) effects only at the second phase of the experiment by 49.3% and 53.7%, respectively. Naloxone (5 mg/Kg, *i.p.*) was not able to revert the extract effect, however caffeine (10 mg/Kg, *i.p.*) reverted its effect by 19.8% at the second phase of the formalin test. The aqueous extract (200 mg/Kg, *p.o.*) inhibited edema by 12.9% when we used the rat paw edema model. The acute toxicity was low in mice.

Conclusion: The *T. avellanedae* inner bark aqueous extract presented antinociceptive and antiedematogenic activities at the used models, with a possible antinociceptive effect associated to the adenosine system.

Background

Tabebuia avellanedae is a tree from the *Bignoniaceae* family. Commonly known as "pau d'arco" in Brazil, its in-

ner bark is used as analgesic, anti-inflammatory, antineoplastic [1,2] and diuretic at the Brazilian northeast. Its anti-inflammatory, antimicrobial, and antineoplastic ac-

tivities are cited in the literature as promoted by saponines, flavonoides, cumarines, and natural antibiotics, such as the lapachol and its derivatives encountered on its constituents [3–6]. The antifungal activity of aqueous, dichloromethane and methanol extracts from *T. avellanedae* and other 13 Paraguayan plants were accessed by Portillo *et al.* [7]. The aqueous and methanol extracts of *T. avellanedae* showed the highest antifungal activity. Phytochemical studies revealed the presence of naphthoquinones, anthraquinones and quinoid compounds in *T. avellanedae*, although lapachol, previously cited as anti-inflammatory [8], was not detected in its aqueous extract [9]. Since *T. avellanedae* inner bark is extensively used as analgesic in Brazil, the goal of our work was to describe the antiedematogenic and antinociceptive effects and acute toxicity of *T. avellanedae* inner bark aqueous extract on various animal models.

Results

The inhibition of rat paw edema induced by carrageenan was significantly different between all groups (ANOVA: $F_{0.05;3;140} = 45.72$; $p < 0.0005$) and time intervals tested (ANOVA: $F_{0.05;4;140} = 35.66$; $p < 0.0005$) The volume variation was not proportional between all groups (ANOVA: $F_{0.05;12;140} = 1.94$; $p < 0.05$). The *T. avellanedae* aqueous extract inhibited edema only at the 200 mg/Kg dose (Tukey: $q_{0.05;140;4} = 5.02$; $p < 0.05$, Table 1). This test is used to evaluate anti-inflammatory drugs and has been used to test the antiedematogenic effect of various substances [10].

When performing an writhing test, results showed notable differences at the writhing frequency between all tested groups (Kruskal-Wallis: $F_{0.05;5;47} = 9.26$; $p <$

0.0005). *T. avellanedae* (100, 200 and 400 mg/Kg, p.o.) reduced the acetic acid effect (Nemenyi: $q_{0.05;\infty;6} = 4.28$, $p < 0.05$ and $q_{0.05;\infty;6} = 4.17$, $p < 0.05$, respectively, Table 2). The abdominal constriction response is an unspecific nociception model used to evaluate the central and peripheral analgesics drug activities [11].

Our experiments showed significant differences between four groups when the reaction time was taken into account at the formalin 1% test during the first and second phases (Kruskal-Wallis: $F_{0.05;4;34} = 22.52$; $p < 0.0005$ and $F_{0.05;4;34} = 23.45$; $p < 0.0005$, respectively). The aqueous extract of *T. avellanedae* (200 e 400 mg/Kg) reduced the formalin effect only at the second phase (Nemenyi: $q_{0.05;\infty;5} = 4.264$, $p < 0.05$ and $q_{0.05;\infty;5} = 4.643$, $p < 0.05$, Table 3).

Naloxone (5 mg/Kg, i.p.) did not revert the action of the aqueous extract (400 mg/Kg) (Nemenyi: $q_{0.05;\infty;3} = 4.27$; $p < 0.05$). Caffeine, an adenosine A_1 and A_2 receptors antagonist, reverted the *T. avellanedae* aqueous extract action at the formalin test second phase (Nemenyi: $q_{0.05;\infty;3} = 1.45$; $p > 0.05$, Table 3). There was no LD₅₀ when using doses up to 5000 mg/Kg.

Discussion

The aqueous extract at 200 mg/Kg inhibited the rat paw edema induced by carrageenan in a similar way as indomethacin (Table 1). However at 400 mg/Kg we did not detect edema reduction. It would be expected a dose-dependent effect when using higher concentrations. Since the aqueous extract contains several compounds, another compound in the extract may be acting simultaneously to revert its own action.

Table 1: Effects of control, *T. avellanedae* aqueous extract (AE, 200 and 400 mg/Kg), and indomethacin (10 mg/Kg) at the rat paw edema induced by carrageenan 1%. The paw volume was measured four times with 1 h intervals between them. It is shown these intervals and the inhibition percentages for each interval.

Treatment (p.o.)	Dose (mg/Kg)	Average \pm SE (mL)							
		1 h	%	2 h	%	3 h	%	4 h	%
Control	-	1.33 \pm 0.06	-	1.60 \pm 0.14	-	1.69 \pm 0.12	-	1.7 \pm 0.12	-
Indomethacin	10	0.82 \pm 0.02 ^a	38.6	0.88 \pm 0.02 ^a	45.0	0.97 \pm 0.06 ^a	42.5	1.0 \pm 0.07 ^a	41.3
AE	200	1.13 \pm 0.06 ^a	15.6	1.33 \pm 0.06 ^a	16.7	1.47 \pm 0.05 ^a	12.7	1.46 \pm 0.05 ^a	14.0
AE	400	1.2 \pm 0.04	9.8	1.5 \pm 0.07	6.7	1.65 \pm 0.08	2.3	1.61 \pm 0.08	5.4

%, edema inhibition percentage (n = 8/group). SE, average standard error. ^ap < 0.05 compared to control (Anova, followed by Tukey).

Table 2: Effects of control, *T. avellanedae* aqueous extract (AE, 100, 200, and 400 mg/Kg), indomethacin (10 mg/Kg), and morphine (2.5 mg/Kg) at the writhes induced by acetic acid 0.6%. The number of writhes median is shown in the third column, followed by inhibition percentages.

Treatment	Dose (mg/Kg)	Median	%
Control	-	29	-
Indomethacin (p.o.)	10	2 ^a	65.1
Morphine (i.p.)	2.5	3 ^a	52.6
AE (p.o.)	100	7 ^a	44.9
AE (p.o.)	200	3 ^a	63.7
AE (p.o.)	400	4 ^a	43.8

n = 9 per group. %, writhing inhibition percentage. ^ap < 0.05, compared to control (Kruskal-Wallis, followed by Nemenyi).

Although the abdominal writhes induced by acetic acid represent a peripheral nociception model [12], this is not a specific model, since several compounds, such as, tricyclic antidepressants [13] and anti-histaminic [14] inhibit the writhes induced by acetic acid. Even though the *T. avellanedae* AE prevented the writhes, there is still the need for studies using other analgesia models.

Three distinct phases are involved on the acute vascular response. At the first phase simultaneous release of histamine and serotonin occurs. At the second phase kinin release, such as bradykinin, occurs and at the terminal phase prostaglandins release occurs [15]. Any substance

inhibiting the carrageenan action at the used model is considered as having anti-inflammatory action.

Since the effect of the AE was not reverted by naloxone at the formalin test second phase, our studies indicate that the opioid system does not participate at the AE (200 and 400 mg/Kg) pharmacological properties. On the other hand caffeine reverted the AE action at the formalin test second phase, suggesting the participation of the adenosine system at the analgesic effect. Adenosine may interact with at least four subtypes of receptors coupled with protein G, namely A₁, A_{2a}, A_{2b}, and A₃[16]. There has been evidences that activation of the A₁ and A₂ receptors produce an antinociceptive effect, while activation of the A₃ receptor produce nociception [17]. These facts are supported by experiments where caffeine produced antinociception [16]. Henceforth the AE antinociceptive effect at the formalin test second phase may be associated to an activation of the A₁ and/or A₂ adenosine receptors. Further studies are needed to confirm these findings.

The formalin test is one of the most used models to explain pain and analgesia mechanisms, with better results than the ones using mechanical or thermal stimulus [18]. This model is constituted by two distinct phases. The first phase represents the irritating effect of formalin at the sensorial fibers-C. The second is an inflammatory pain response. Central acting analgesics, such as morphine, inhibit both phases. Peripheral acting drugs, such as non-steroid anti-inflammatory and corticosteroids, inhibit only the second phase [19].

Table 3: Effect of *T. avellanedae* aqueous extract (AE, 100, 200, and 400 mg/Kg) and morphine at the formalin 1% test. The results of the AE at the analgesia model induced by formalin are shown as the median of the time, which each mouse spent licking its posterior left paw after administration of formalin (1%) and its respective percentage. The AE (200 and 400 mg/Kg) significantly reduced the second phase of the formalin test.

Treatment	Dose (mg/Kg)	1 st phase		2 nd phase	
		Median	%	Median	%
Control	-	58.5	-	36	-
Morphine (i.p.)	7.5	10.5 ^a	87	0 ^a	81.8
AE (p.o.)	100	45	38	16	28.5
AE (p.o.)	200	43	43.1	6 ^a	49.3
AE (p.o.)	400	38	43.1	1 ^a	53.7
Morphine (i.p.)+Naloxone (i.p.)	5	55.5	16.5	18 ^a	33.8
Naloxone (i.p.)+AE400	5	37 ^a	62.8	3 ^a	71.4
Caffeine (i.p.)+AE400	10	44 ^a	55	26.5	19.8

%, pain reaction time inhibition percentage. ^ap < 0.05 compared to control (Kruskal-Wallis, followed by Nemenyi), AE400, aqueous extract 400 mg/Kg.

It was not possible to measure the plant LD₅₀ due to the lack of mice deaths even at high concentration of the *T. avellanedae* aqueous extract. This result indicates that the plant has a low toxicity profile [20]. The plant presented antiedematogenic and antinociceptive at the peripheral system, probably due to its action at mediators, such as bradykinin and prostaglandins at the neuron termination. Hiperalgesy may also be involved on this process, mediated by the adenosine A₂ receptor [21,22].

Conclusion

Tabebuia avellanedae is used by popular medicine as anti-inflammatory, antimicrobial, antineoplastic, and diuretic. The *T. avellanedae* inner bark aqueous extract presented antinociceptive and antiedematogenic activities at the used models, with the antinociceptive effect associated to the adenosine system. These results validate the plant popular use as analgesic and antiinflammatory. The antimicrobial, antineoplastic, and diuretic activities were not studied.

Materials and Methods

Plant material

The *Tabebuia avellanedae* inner bark collected at the "Capim Grosso" village (Canindé do São Francisco region) was used. The plant identification was made on site by our herbalist (Gilvane V. Souza, Biology Department) and a voucher specimen number 2955 was deposited at the University of Sergipe herbarium, (Universidade Federal de Sergipe, CCBS, Departamento de Biologia, São Cristóvão, Sergipe, 49100-000, Brazil).

Aqueous extract preparation

The plant inner barks were dried at 40°C in oven and triturated using a mill in order to obtain a powder. Distilled water (1:10 w/v) at 100°C was added to the triturated powder, constituting the aqueous extract. The extract was infused for 30 min, filtered, lyophilized and used on the pharmacological tests. The yield of the aqueous extract was 8.9% w/w. In order to perform all experiments the extract was reconstituted in enough water to make a 100 mg/mL solution and administered p.o. 60 minutes before the experiment.

Animals

Swiss mice (20–35 g) and Wistar rats (120–200 g) male and female were used as test animals. The animals were maintained in plastic boxes, with food and water *ad libitum*. The animals submitted to oral administration of the extract or drugs were fasted for 12 hours.

Drugs preparation

Drugs used in the experiments were diluted in a way to obtain a injection volume of 0.1 mL/10 g (animal weight), except when defined in the text. Each drug was

dissolved in appropriate solvents as follows: Indomethacin (Sigma), diluted in water/0.1 N NaOH (pH = 8); carrageenan 1% (Sigma), in saline solution; acetic acid 0.6% (Merck), in water; Morphine hydrochloride (Sigma) in water; formalin 1% (Baker) in water; naloxone hydrochloride (Sigma), in water; caffeine (Sigma) in water.

Acute toxicity (LD₅₀)

In order to verify the plant LD₅₀, mice (n = 10) received the aqueous extract (1, 3 and 5 g/Kg; p.o.). The mortality index was observed during 48 hours [20].

Rat paw edema test

The antiedematogenic activity was evaluated by the rat paw edema test [10] (n = 8) induced by carrageenan at 1% in. Indomethacin (10 mg/Kg; p.o.) and the *T. avellanedae* aqueous extract (200 and 400 mg/Kg; p.o.) were administered 1 h before the flogistic agent subplantar injection. The control group received only the carrageenan injection. The paw volume was measured at the time 0, then measured again 1, 2, 3, and 4 h after the carrageenan administration by the water displacement method measured with the help of a plethysmometer (model 7150, Ugo Basile).

Writhing test

The antinociceptive effect was evaluated by the writhing test [11] in mice (n = 9), induced by acetic acid 0.6% (0.1 mL/10 g; i.p.). The *T. avellanedae* aqueous extract (100, 200 and 400 mg/Kg; p.o.) was administered 1 h before the nociceptive agent. Ten minutes after the acid administration we observed the number of writhes for a period of 20 min. Morphine (2.5 mg/Kg, i.p.) and indomethacin (10 mg/Kg, p.o.) were used as test standards.

Formalin test

The antinociceptive effect was also evaluated by the formalin test. It was observed the time (seconds), which each mice (n = 8) spent licking its posterior left paw after administration, subplantar route, of formalin 0.02 mL, 1% [19,23]. The *T. avellanedae* aqueous extract (100, 200 and 400 mg/Kg; p.o.) was administered 1 h before the formalin injection. The standard test drug was morphine (7.5 mg/Kg, i.p.). The reaction time to pain was measured during 0–5 min and 20–25 min after the stimulus. In order to verify a possible opioid system participation, naloxone (5 mg/Kg; i.p.) was administered with the AE to another animal group (n = 8, Table 3). In a similar way caffeine (10 mg/Kg; i.p.) was administered with the AE to verify possible adenosine system receptor participation. Morphine and naloxone were also administered to another animal group.

Statistical analysis

The inflammation test results were analyzed by ANOVA followed by the Tukey test. In order to analyze the nociception model we used the non-parametrical analogous test, the Kruskal-Wallis followed by the Nemenyi test, with a significance level of 5%. Inhibition percents were calculated by the formula: Inhibition percent = $(1 - Vt/Vc) \times 100$, where Vt and Vc represent the average paw volume, the number of writhes, or the licking paw time of the treated and control groups, respectively.

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