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Effects of *Lecythis pisonis* Camb. (Lecythidaceae) in a mouse model of pruritus

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ABSTRACT

Ethnopharmacological relevance: Lecythis pisonis Camb. (Lecythidaceae), is popularly known as "Sapucaia". In traditional medicine, leaves are used for the treatment of pruritus.
Aim of the study: The present study is aimed to investigate the antipruritic effect of the ethanol extract from leaves of *Lecythis pisonis* (LPEE), fractions (hexane-LPHF, ether-LPEF and ethyl acetate-LPEAF) and mixture of triterpenes [ursolic and oleanolic acids (MT)] in mice and rats.
Materials and methods: The LPEE, LPHF, LPEF, LPEAF and MT were evaluated on scratching behavior induced by compound 48/80 in mice. In addition, LPEE, LPEF and MT were investigated on rat peritoneal mast cells degranulation induced by compound 48/80 (*ex vivo* study). The anti-inflammatory activity of LPEE and LPEF was investigated in rats using carrageenan-induced hind paw edema model. In the evaluation of the spontaneous motor activity, the LPEE was studied for its effect on spontaneous motor activity in an open-field test in mice.
Results: The scratching behavior induced by compound 48/80 was significantly inhibited in mice pretreated with LPEE, LPHF, LPEF, LPEAF and MT. The suppressive effect of LPEE, LPEF and MT was only partially antagonized by naloxone. In addition, the compound 48/80-elicited degranulation of rat peritoneal mast cells was also markedly reduced in animals pretreated with LPEE, LPEF and MT. In the anti-inflammatory test, LPEE decreased the paw edema at the third hour after carrageenan (Carr) administration. Moreover, LPEF also was able to inhibit the oedematogenic response evoked by carr at all analysed time points. In the open-field test, LPEE-pretreated mice showed no impairment of spontaneous locomotion. Furthermore, the LPEE demonstrated no overt toxicity up to an oral dose of 2 g/kg in an acute toxicity assay.
Conclusions: These results clearly indicate the antipruritic effects of *Lecythis pisonis* leaves and suggest that this effect may be related to a stabilizing action on mast cell membrane. Furthermore, these data support the traditional use of this plant against cutaneous pruritus.

1. Introduction

Pruritus is an unpleasant sensation related to many skin diseases like contact dermatitis, atopic dermatitis and urticaria. In these diseases, itching aggravates the skin lesions and the development of dermatitis (Ueda et al., 2006). Thus, the most effective strategy to prevent worsening of skin lesions and improve life quality of patients with dermatitis is to reduce the pruritus (Caroline, 1999). Many endogenous chemical agents such as amines, proteases, growth factors, neuropeptides, opioids, eicosanoids and cytokines can act as pruritogens causing histamine release from mast cells and/or by raising awareness of C-fibers (Lerner, 1994; Hagermark, 1995; Schmelz et al., 1997). Histamine receptors are known to mediate histamine-induced responses, and are members of the G-protein-coupled receptors. Four histamine receptors subtypes have been identified to date (Rossbach et al., 2011), and histamine receptor subtype I (H1R) has been most extensively studied in the context of histamine-induced itch. In fact, H1R blockers (antihistamines) are widely used to manage and relieve itch symptoms (Simons et al., 1984). However neither type of antihistaminics offers sufficient relief of most pruritus, such as topic dermatitis, chronic cholestase and to date no drug, effective in the full spectrum of pruritus, has been described.

Lecythis pisonis Camb., belong to the *Lecythidaceae* family, popularly known as "Sapucaia" or "Cumbuca de Macaco". It is a large tree with rough bark deeply fissured and grayish color (Braga, 1953) widely distributed the states of Piaui, from Pernambuco to Sao Paulo and in the Amazon region (Mori and Prance, 1990). The seeds of this species are a valuable source of essential amino acids, fatty acids and minerals (Vallilo et al., 1999) being a functional and

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nutritious food for human consumption (Denadai et al., 2007). In traditional medicine, the leaves are used as a bath, for the treatment of itching (pruritus) in the body (Franco and Barros, 2006) and the oil extracted from the seeds is used as an emollient, reducing muscle pain (Agra et al., 2007).

Phytochemical investigation of ethanolic extract of *Lecythis pisonis* leaves has led to the identification of different fraction such as hexane, ether and ethyl acetate. However, the chromatographic fractioning of the ethereal fraction and its subfractions spectrometric analysis resulted in the identification of 10 isoprenic origin substances, distributed in three binary mixtures of triterpenoid skeletons and ursano oleanano, a mixture of steroid and triterpenoid skeleton called friedelan-3β-ol. The presence of triterpenes in the species was confirmed by the isolation and identification of the mixture of ursolic and oleanolic acids (Oliveira, 2010).

Based on literature search, no scientific report has been published on the potential antipruritic activity of *Lecythis pisonis* despite the traditional uses of the plant to treat of pruritus. Thus, the present study is aimed, for the first time, to evaluate the antipruritic potential of the ethanol extract, fractions and mixture of triterpenes of the leaves of *Lecythis pisonis* using murine model of pruritus.

2. Materials and methods

2.1. Plant material and identification

Leaves of *Lecythis pisonis* Camb. (Lecythidaceae) were collected in July 2008 at the Centre for Agrarian Sciences, Federal University of Piauí (CCA-UFPI) in the city of Teresina, Piauí state, Brazil (5°02′ 53.2″S, 42°47′ 16.8″O to 68 m sea level). After collection, a voucher specimen was identified by Gardene M. Sousa and deposited in the Graziela Barroso Herbarium of Federal University of Piauí (TEPB 26488).

2.2. Extraction and isolation

Dried and powdered leaves of *Lecythis pisonis* (2 kg) were extracted at room temperature, consecutively six times with ethanol 98%. The solvent was removed by evaporation under reduced pressure using Hedolph Rotary Evaporator to yield the ethanolic extract (LPEE, 272.0 g, 13.6%). Part of the extract (200.0 g) was fractionated by serial extraction with hexane, ethyl ether, AcOEt, and H_2O to yield hexane (LPHF, 60.0 g, 34.0%), ethyl ether (LPEF, 24.0 g, 12.0%), EtOAc (LPEAF, 21.0 g, 10.5%) and H_2O (LPAF, 70.0 g, 37.5%) fractions and a precipitate formed in the EtOAc phase which was separated by simple filtration (ppt-EtOAc, 10 g, 5%). The ethereal fraction (LPEF, 10 g) was fractionated on a silica gel column with elution gradient from $CHCl_3$ to MeOH to yield 103 fractions collected as follows: 1–31 ($CHCl_3$, 100%), 32–54 ($CHCl_3$–MeOH, 98:2), 55–62 ($CHCl_3$–MeOH, 95:5), 63–72 ($CHCl_3$–MeOH, 9:1), 73–89 ($CHCl_3$–MeOH, 8:2), 90–96 ($CHCl_3$–MeOH, 7:3), 97–103 (MeOH 100%). The fraction B (17–23, 603 mg) was suspended in MeOH to form an amorphous precipitate (F17-ppt, 475 mg, 4.75%) composed of a mixture of ursolic and oleanolic acid triterpenes (Fig. 1). The structural characterization of this mixture was performed using spectroscopic methods such as Nuclear Magnetic Resonance (^1H and ^{13}C NMR) and compared with literature data (Mahato and Kundu, 1994; Junges et al., 2000). The ratio of ursolic acid and oleanolic acid in this mixture was 59:41, calculated by ^1H NMR, by dividing the signal area of olefinic hydrogens $\delta = 5.15$ (ursolic acid) and $\delta = 5.19$ (oleanolic acid) with the signal area of $\delta = 3.11$ (dd, $J = 7.0$ and 9.0 Hz) attributed to H-3 in the two triterpenes and multiplying by 100 (Oliveira, 2010).

The ^1H and ^{13}C NMR spectra were recorded on a Varian Inova 500 spectrometer, in $CDCl_3$, at 500 and 125 MHz, respectively, using

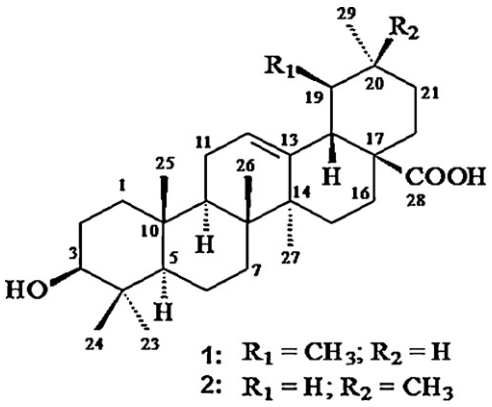

Fig. 1. Chemical structures of ursolic acid (1) and oleanolic (2) acid.

TMS as internal standard. The chemical shifts values are on δ scale and the coupling constants (J) values are in Hertz. Column chromatography was carried out using silica gel 60 (0.063–0.200 mm). Thin Layer Chromatography (TLC) was carried out on silica gel 60 G (Merck) plates (0.25 mm layer thickness).

Extract, fractions and mixture of triterpenes of *Lecythis pisonis* leaves were dissolved in or diluted with physiological saline (0.9%) and prepared immediately before each experiment. The triterpenes concentration of the extract, fractions and mixture were adjusted to a volume of 10 mL/kg.

2.3. Chemicals and reagents

Compound 48/80, cyproheptadine, naloxone, ketotifen and carrageenan were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Diazepam and morphine were purchased from Cristália Produtos Químicos e Farmacêuticos Ltd. (SP, Brazil).

2.4. Animals

Male Swiss mice 25–30 g ($n = 5$–8) were used, 10 weeks old and male and female Wistar rats 180–220 g ($n = 5$–8), 3 months old from the Sector Animal House for Research on Medicinal Plants of the Federal University of Piauí. The animals were kept at 24 ± 1 °C with a light/dark cycle of 12 h, with free access to food and water. They were fasted for a period of 18 h and were accustomed to the test environment for 1 h before each experiment. All experimental protocols were approved by the Ethics Committee for Animal Experimentation at the Federal University of Piauí (No. 084/2010).

2.5. Acute toxicity

Groups of 10 mice (5 males and 5 females) were used. The control group received only vehicle, and the remaining groups received increasing doses up to 2 g/kg of the LPEE, orally. Animals were maintained in a cage with free access to a standard diet and water *ad libitum* and they were observed for up to 14 consecutive days. Besides the number of deaths, parameters such as alertness, sedation, ptosis, dyspnea, urination, diarrhea, convulsions, spontaneous motor activity, postural reflex, piloerection, nociception and others (aggression, grooming, rearing, climbing and vocalization), were observed for 72 h.

2.6. Antipruritic activity

2.6.1. Scratching behavior induced by compound 48/80

One day before the experiments the rostral part of the skin on the back of each mouse was clipped. On the test day, the animals (male

Swiss mice, $n = 8$ per group) were first accustomed for about 10 min in the observation plastic cages (10 cm × 15 cm × 30 cm), after that they were treated orally with vehicle (saline 0.9%, 0.1 mL/10 g), LPEE (50, 100, 200 mg/kg), LPHF, LPEF, LPEAF (50, 100, 200 mg/kg), MT (12.5, 25 and 50 mg/kg) or cyproheptadine (10 mg/kg, 1 mg/mL). One hour after treatment the animals received a subcutaneous injection of compound 48/80 (100 μg/100 μL) in the dorsal region of the head using a 27 gauge needle. Control animals received the same volume of saline 0.9%. Right after the injection the mice were returned to their observation cages, one mouse per cage. The scratching behavior was observed for 20 min by observers who were unaware of the treatments (Ishiguro and Oku, 1994) and expressed as the time in seconds (s) that each animal had spent scratching during a 20-min period. This period was chosen based on our pilot studies that showed increased intensity of itching in the time-course response.

Only scratching of nose by fore- or hind paws and the injection site by hind paws was counted in animal group that received the compound 48/80 (Kuraishi et al., 1995). The dosage selection of pruritogen agent was chosen based on our pilot studies and was able to induce a consistent scratching during the first 20 min.

2.6.2. Effect of μ-opioid receptor antagonist naloxone on the compound 48/80 induced scratching behavior in mice pretreated with ethanol extract, ether fraction and of triterpenes mixture (oleanolic and ursolic acids) or morphine

To evaluate the possible role of endogenous opioids in the antipruritic activity of LPEE, LPEF and MT against compound 48/80-indeced scratching, groups of male Swiss mice ($n = 8$ per group) were pretreated with vehicle (0.9% saline, 0.1 mL/10 g, p.o.), LPEE (200 mg/kg, p.o.), LPEF (200 mg/kg, p.o.) and MT (50 mg/kg, p.o.) 1 h before and naloxone, a *non-selective* μ-opioid receptor *antagonist* (2 mg/kg, 0.2 mg/mL, i.p.), or morphine (5 mg/kg, 0.5 mg/mL, s.c.) 30 min before subcutaneous administration of compound 48/80 (100 μg/100 μL) in the dorsal region of the head. Control animals received the same volume of saline 0.9% (Kuraishi et al., 1995).

2.6.3. Peritoneal mast cell degranulation

This *ex vivo* study aimed to demonstrate the effect of LPEE, LPEF, MT and ketotifen on the mast cells degranulation. Male Wistar rats ($n = 5$ per group) were treated with saline (0.9%, 10 mL/kg, p.o.), vehicle (saline 0.9%, p.o.), LPEE (200 mg/kg, p.o.), LPEF (200 mg/kg, p.o.), MT (100 mg/kg, p.o.) or ketotifen (1 mg/kg, 0.1 mg/mL, p.o.). After 1 h, the animals were killed by cervical dislocation and half a centimeter pieces of mesenteric vascular plexus were collected from the respective groups into each of the glass tubes containing Ringer Locks fluid (10 mL). The mast cell degranulation was induced by incubation of the mesentery, in the respective groups, with compound 48/80 (final concentration of 0.4 mg/mL). The concentration of 0.4 mg/mL of the compound 48/80 was based on preliminary tests, which caused degranulation of mast cells around 90% extension. The same volume of distilled water was added to test tubes containing the mesentery of animals that received only saline. After 30 min of incubation, the mesenteries were mounted on glass slides and stained with 0.1% toluidine blue for observation of degranulated mast cells in optic microscopy. The counting of intact and degranulated mast cells was performed in five fields of each slide and the result expressed as the percentage of degranulated mast cells (Norton, 1954).

2.7. Antiedematogenic activity

2.7.1. Carrageenan-induced hind paw edema

Both sexes Wistar rats ($n = 8$ per group) of were treated with vehicle (0.9% saline, 10 mL/kg, p.o.), LPEE (50, 100, 200 mg/kg, p.o.), LPEF (50, 100, 200 mg/kg p.o.) or indomethacin (10 mg/kg, 1 mg/mL, p.o.) 60 min before injection of carrageenan (1%, 0.1 mL, i.pl.) in the sub-plantar region of the animal right hind leg. After 1, 2, 3, 4, 5 and 6 h from the carrageenan administration, the paw diameter was determined using a digital micrometer (DIGIMESS). The difference in diameter of paw edema was calculated for each group and compared with the control group (Winter et al., 1962).

2.8. Evaluation of the spontaneous motor activity

The motor activity of animals was verified by an open field, square (30 cm × 30 cm × 15 cm), with its base divided into nine squares of equal diameters. Male Swiss mice ($n = 5$ per group) were treated with vehicle (0.9% saline, 10 mL/kg, p.o.), LPEE (50, 100 and 200 mg/kg, p.o.) or diazepam (4 mg/kg, 0.4 mg/mL, i.p.). After 45 min of administration, the animals were taken individually to the open field and observed for a period of 4 min, with 1 min of adaptation, and the frequency of locomotion, based on the model described by Capaz et al. (1981).

2.9. Statistical analysis

The values were expressed as mean ± S.E.M. All data were submitted to variance analysis (ANOVA) one-way, followed by Tukey test. Values of ($p < 0.05$) were considered statistically significant. GraphPad Prism® 4.0 Software was used.

3. Results

3.1. Effect on scratching behavior induced by compound 48/80

The group of mice previously treated with saline did not exhibit the itching behavior (date not shown), while animals in the group receiving subcutaneous injection of compound 48/80 (100 μg/100 μL) into the rostral back, showed intense scratching.

Mice that received 100 and 200 mg/kg LPEE, LPHF or LPEF exhibited significant ($p < 0.001$) inhibition of compound 48/80 induced scratching (45.82%, 63.24%, 43.34%, 58.28%, 31.96% and 77.31%, respectively) as compared to respective control groups. At 50 mg/kg there were no significant effect (Fig. 2A, B and C). The scratching behavior was less prominent in mice treated with LPEAF (100 and 200 mg/kg) (21.12% and 37.64%, respectively) as compared to control group (Fig. 2D). The oral treatment with MT (25 and 50 mg/kg) was also capable of reducing the itch induced by administration of compound 48/80 (35.84% and 59.26%, respectively) (Fig. 2E). Furthermore, the dose of 12.5 mg/kg was not effective in inhibiting the itching compared with the control group. The dual histamine/serotonine receptor antagonist, cyproheptadine (10 mg/kg) also diminished of scratching responses elicited by compound 48/80.

3.2. Effect of μ-opioid receptor antagonist naloxone on the compound 48/80 induced scratching behavior in mice pretreated with ethanol extract, ether fraction and of triterpenes mixture (oleanolic and ursolic acids)

As shown in Table 1, previous treatment with morphine (5 mg/kg, s.c.), LPEE (200 mg/kg, p.o.), LPEF (200 mg/kg, p.o.) and MT (50 mg/kg, p.o.) resulted in significant suppression of compound 48/80 induced scratching behavior in mice with inhibition percentage of 94.64%, 57.48%, 80.83% and 54.71%, respectively. Even though naloxone (2 mg/kg, i.p.), a non-selective antagonist of opioid receptors, alone showed no significant influence that could reverse the morphine effect. The suppressive effect of LPEE, LPEF and MT was only partially antagonized by naloxone.

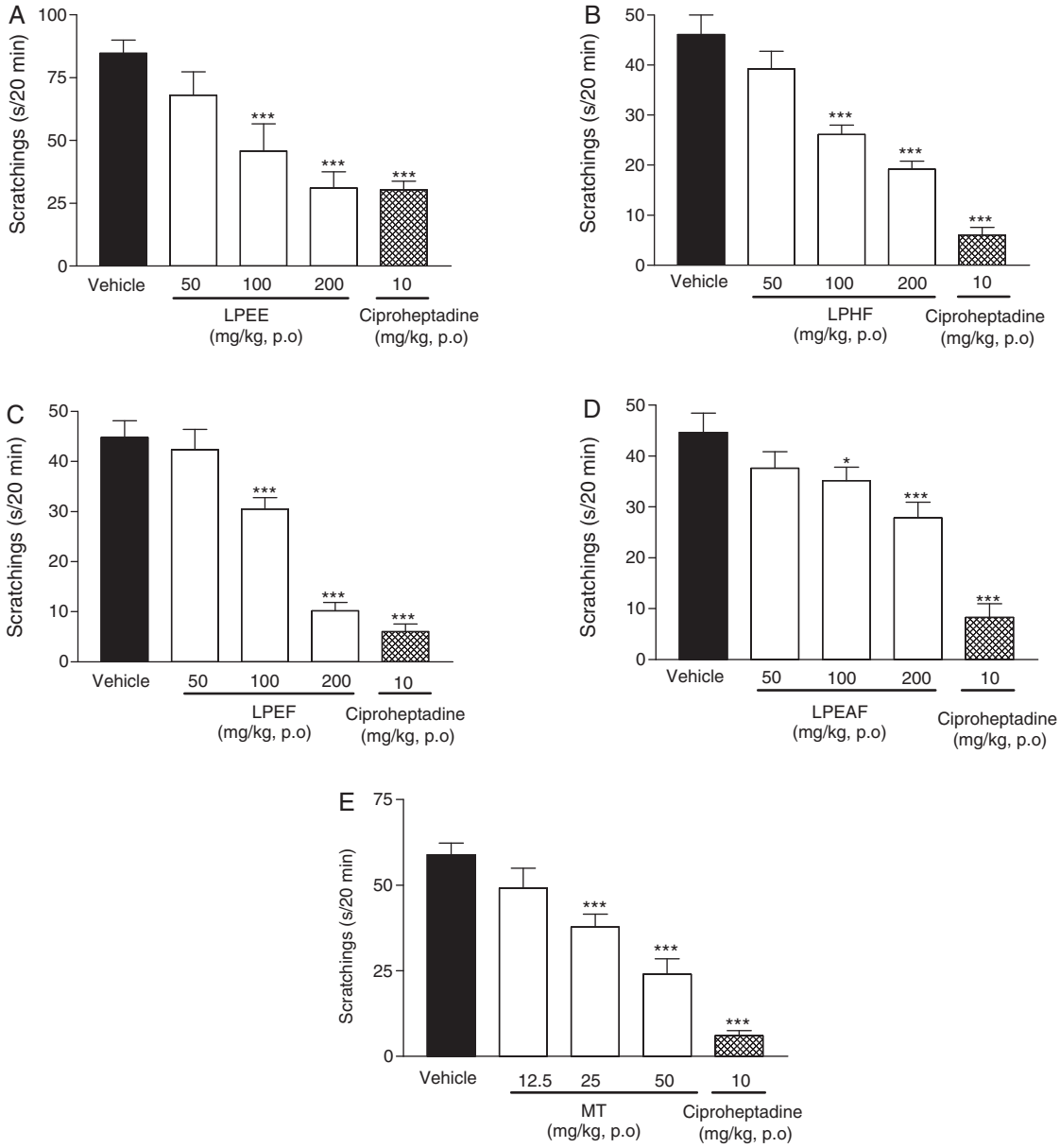

Fig. 2. Effect of ethanolic extract of *Lecythis pisonis* leaves – LPEE (A), hexane fraction – LPHF (B), ether fraction – LPEF (C), ethyl acetate fraction – LPEAF (D) and the mixture of ursolic and oleanolic acids – MT and (E) administered orally on the scratching behavior induced by compound 48/80 in mice. Each column represents the mean ± S.E.M. of 5–8 animals. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) vs. vehicle (one-way ANOVA followed by Tukey test).

3.3. Effect on peritoneal mast cell degranulation

Table 2 shows the effect of LPEE, LPEF and MT on peritoneal mast cell degranulation induced by compound 48/80 (*ex vivo*). Compared with vehicle-treated controls, treatment with LPEE (200 mg/kg, p.o.), LPEF (200 mg/kg, p.o.), MT (50 mg/kg, p.o.) and ketotifen (1 mg/kg) significantly reduced the compound 48/80-induced degranulation in 31.26%, 49.12%, 55.80% and 80.41%, respectively. A representative microphotographs showing the mast cell degranulation among various groups are shown in Fig. 3.

3.4. Effect on spontaneous locomotor activity

The ethanolic extract (LPEE) (50, 100 and 200 mg/kg) did not affect the locomotion frequency in mice. The observed locomotion frequencies in the fraction-treated groups, were not statistically different from those of vehicle-treated control group over a 5-min period. Only diazepam (4 mg/kg, i.p.) significantly decreased the number of quarters invaded in comparison with control group (Table 3).

3.5. Effect on carrageenan-induced hind paw edema

The anti-edematogenic response obtained by administration of the ethanolic extract (LPEE) and partitioned fraction (LPEF, ethereal fraction) of the leaves of *Lecythis pisonis*, indometacin and vehicle on carrageenan-induced hind paw edema in rats is shown in Tables 4 and 5. Interplantar injection of carrageenan in rats caused a local inflammatory response; this increase was observed at 1 h and peaked 3 h after the phlogistic agent application. Administration of LPEE (100 and 200 mg/kg, p.o.) 1 h before injection carrageenan caused a significant ($p < 0.05$) inhibition of increase in paw edema in the 1st (17.22% and 16.87%), 2nd (20.43% and 8.22%) and 3rd (16.33% and 17.21%) hour when compared to vehicle control.

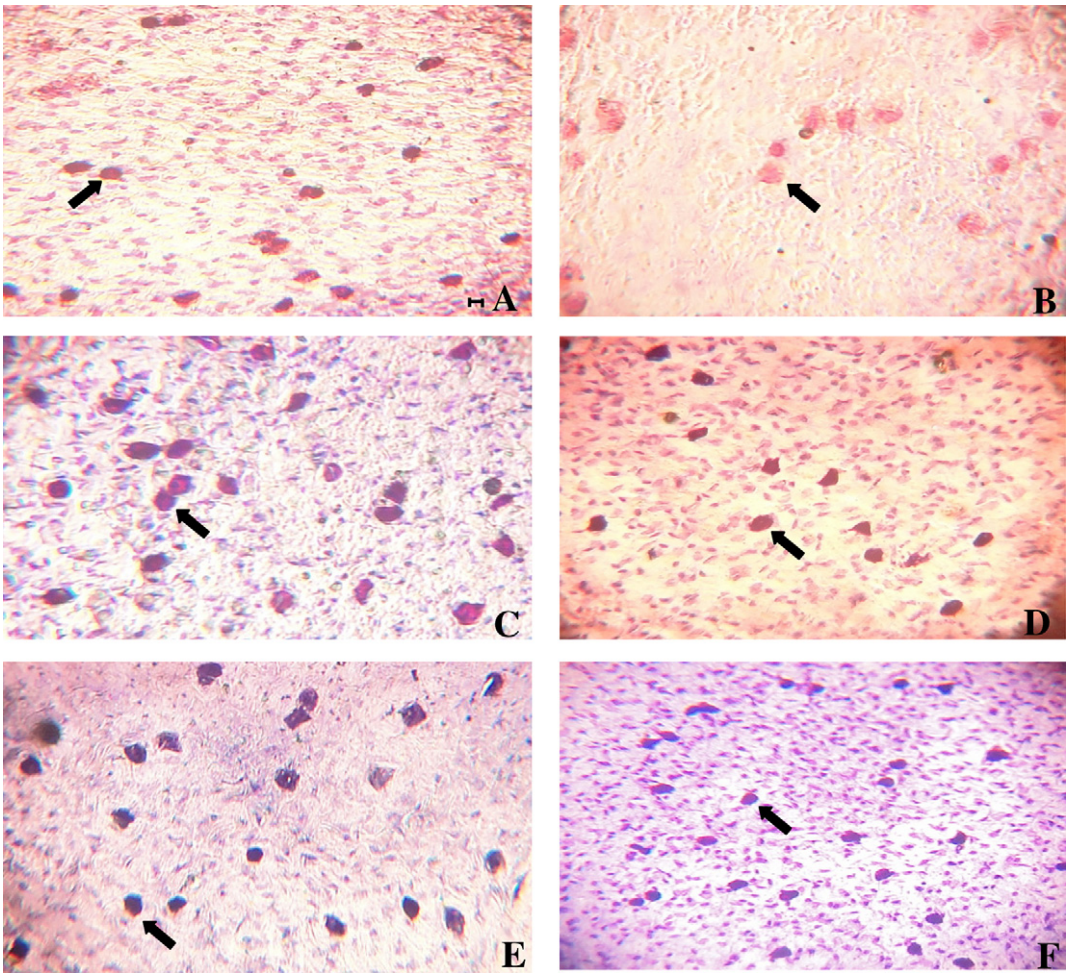

Fig. 3. The representative microphotograph of toluidine blue stained rat peritoneal mast cells (some indicated by solid arrowheads) subjected or not to compound 48/80 (0.4 μg/mL)-induced degranulation *ex vivo*. (A) Mast cells from saline-treated rats; (B) mast cells from vehicle (saline 10 mL/kg) treated rats incubated with compound 48/80, showing extensive mast cell degranulation; (C) mast cells from ethanolic extract (LPEE, 200 mg/kg), (D) mast cells from ether fraction (LPEF, 200 mg/kg), (E) mast cells from mixture of ursolic and oleanolic acids (MT, 100 mg/kg) and (F) ketotifen (1 mg/kg) treated rats incubated with compound 48/80, showing inhibition of degranulation (magnification 200×, scale bar = 50 μm).

Table 1
Effect of μ-opioid receptor antagonist naloxone on the compound 48/80 (1 mg/mL, s.c.)-induced scratching behavior in mice pretreated with LPEE, LPEF, MT or morphine.

Treatment	Dose (mg/kg)	Scratchings (s/20 min)
Vehicle (p.o.)	–	60.14 ± 7.62
LPEE (p.o.)	200	25.57 ± 3.77[a]
Morphine (s.c.)	5.0	3.22 ± 1.63[a]
Naloxone (i.p.)	2.0	48.12 ± 5.74
Naloxone (i.p.) + LPEE (p.o.)	2.0 + 200	49.16 ± 8.82[b]
Naloxone (i.p.) + morphine (s.c.)	2.0 + 5.0	56.87 ± 8.41[c]
Vehicle (p.o.)	–	53.00 ± 3.16
LPEF (p.o.)	200	10.16 ± 1.70[a]
Morphine (s.c.)	5.0	3.22 ± 1.63[a]
Naloxone (i.p.)	2.0	48.12 ± 5.74
Naloxone (i.p.) + LPEF (p.o.)	2.0 + 200	42.80 ± 2.80[b]
Naloxone (i.p.) + morphine (s.c.)	2.0 + 5.0	55.00 ± 6.37[c]
Vehicle (p.o.)	–	53.00 ± 3.16
MT (p.o.)	50	24.00 ± 4.50[a]
Morphine (s.c.)	5.0	3.22 ± 1.63[a]
Naloxone (i.p.)	2.0	43.57 ± 4.04
Naloxone (i.p.) + MT (p.o.)	2.0 + 50	45.33 ± 3.10[b]
Naloxone (i.p.) + morphine (s.c.)	2.0 + 5.0	55.00 ± 6.37[c]

Data as means ± S.E.M.; $n = 8$ per group; vehicle.
[a] $p < 0.001$ vs. vehicle control.
[b] $p < 0.01$ vs. LPEE, LPEF, MT.
[c] $p < 0.001$ vs. morphine.

Table 2
Effect of ethanolic extract *of Lecythis pisonis* leaves (LPEE), ethereal fraction (LPEF) and the mixture of ursolic and oleanolic acids (MT) on the degranulation of mast cells *ex vivo* induced by compound 48/80 in rats.

Treatment	Dose (mg/kg)	Mast cells disrupted/degranulated (%)
Control (saline)	–	7.3 ± 3.43[***]
Vehicle (48/80)	–	82.2 ± 4.40
LPEE	200	25.7 ± 8.81[***]
LPEF	200	41.82 ± 4.18[***]
MT	100	36.33 ± 1.40[***]
Ketotifen	1.0	16.1 ± 1.31[***]

Data as means ± S.E.M.; $n = 5$ per group; vehicle.
[***] $p < 0.001$ vs. vehicle (48/80).

Table 3
Effect of ethanolic extract of *Lecythis pisonis* leaves (LPEE) on exploratory activity in mice.

Treatment	Dose (mg/kg)	Number of quarters invaded
Vehicle	–	39.80 ± 8.23
LPEE	50	36.0 ± 2.86
LPEE	100	31.67 ± 3.99
LPEE	200	32.57 ± 6.43
Diazepan	4.0	10.29 ± 2.38[***]

Data as means ± S.E.M.
[***] $p < 0.001$.

Table 4
Antiedematogenic effect of the ethanolic extract of *Lecythis pisonis* leaves (LPEE) in the carrageenan-induced hind paw edema.

Treatment (mg/kg, p.o.)	Paw diameter (mm)					
	1 h	2 h	3 h	4 h	5 h	6 h
Vehicle	1.434 ± 0.098	2.236 ± 0.092	2.718 ± 0.157	2.097 ± 0.144	1.865 ± 0.209	1.190 ± 0.219
LPEE (50)	1.904 ± 0.094	2.320 ± 0.112	3.101 ± 0.139	2.523 ± 0.102	1.856 ± 0.161	1.422 ± 0.131
LPEE (100)	1.187 ± 0.190**	1.779 ± 0.255**	2.274 ± 0.157*	1.994 ± 0.170	1.926 ± 0.235	1.325 ± 0.139
LPEE (200)	1.192 ± 0.070**	2.052 ± 0.182*	2.250 ± 0.067**	2.050 ± 0.112	1.698 ± 0.125	1.537 ± 0.070
Indomethacin (10)	0.214 ± 0.051***	0.634 ± 0.127***	0.243 ± 0.109***	0.379 ± 0.140***	0.475 ± 0.136***	0.280 ± 0.097***

Rats were treated with LPEE 60 min (p.o.) before carrageenan-induced hind paw edema. Data represent the mean ± S.E.M. of 5–8 animals. The symbols report significance level.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

However the LPEF (50, 100 and 200 mg/kg, p.o.) was much better than that LPEE. LPEF (de 200 mg/kg) exhibited a significant reduction ($p < 0.001$) in the carrageenan induced edema in all observations (1–6 h), with inhibition rate at 41.55%, 62.68%, 52.68%, 46.10%, 60.65% and 74.86%, respectively. Similarly, indomethacin administration (10 mg/kg, p.o.), used as positive control, showed significant anti-edematogenic effect observed at all times ($p < 0.001$) (69.68%, 90.67%, 77.03%, 83.87%, 89.74% and 95.94%, respectively).

4. Discussion

The results of this study validate the use of *Lecythis pisonis* leaves in the traditional medicine and shows that the ethanolic extract (LPEE), fractions (hexane-LPHF, ethereal-LPEF, ethyl acetate-LPEAF) and the mixture of triterpenes (ursolic and oleanolic acid-MT) are able to suppress experimental pruritus as evidenced from inhibitory effect on scratching behavior induced by compound 48/80 in mice.

Oral administration of LPEE of *Lecythis Pisonis* leaves, up to the dose of 2 g/kg showed no signs of acute toxicity within 14 days of observation (Litchfield and Wilcoxon, 1949), since it was not possible to calculate the lethal dose 50 (LD50). The absence of toxicity presented by LPEE enabled the establishment of the doses used in this study.

The experimental model used to assess the antipruritic activity of *L. Pisoni*, was the model of scratching behavior induced by compound 48/80 in mice. The compound 48/80 is a known mast cells degranulater and its pruritogenic effect is, in part, due to the release of histamine and serotonin from these cells (Inagaki et al., 1999). This pruritogenic agent is able to evoke itch sensation or itch-associated scratching behavior in mice by either subcutaneous or intradermal injection (Kuraishi et al., 1995; Kubo et al., 1997). Stimulation of mast cells by this substance appears to involve several transduction signals, culminating in histamine secretion. The compound 48/80 activate G proteins, activating D phospholipase in mast cells, via G protein–GTP complex, essential for the activation and secretion of mast cells mediators (Tasaka et al., 1986; Chadi et al., 2000).

The results show that oral administration of LPEE, LPHF, LPFE, LPEAF and MT decreased the scratching behavior induced by compound 48/80 in mice compared to vehicle. This finding suggests that inhibition of scratching behavior induced by *Lecythis pisonis* may have occurred through a histamine-releasing mechanism or H1-antagonic activity. This histamine-releasing mechanism was further supported by the fact that the compound 48/80-elicited degranulation of rat peritoneal mast cells (*ex vivo*) was markedly reduced in animals pretreated with LPEE, LPEF and MT.

It was reported that μ-opioid receptors antagonists as naloxone and naltrexone inhibit the itching associated with passive cutaneous anaphylaxis, and intradermal administration of compound 48/80 and P substance, suggesting an opioid mechanism in the pruritus manifestation (Inagaki et al., 1999; Umeuchi et al., 2003). In the present work, we verified the role of endogenous opioids in the scratching response induced by compound 48/80 and its suppression by LPEE, LPEF and MT. The results demonstrated that morphine showed a pronounced reduction of scratching induced by compound 48/80 and that naloxone, a non-selective opioid antagonist, completely reversed the antipruritic action of morphine and only partially the effects of LPEE, LPEF and MT in this model. These results may suggest that the naloxone sensitive opioids system seems at least in part to be involved in the suppressive effect of LPEE, LPFF and MT on compound 48/80-induced scratching in Swiss mice.

In the peritoneal mast cell degranulation *ex vivo* model, LPEE, LPEF and MT were able to prevent degranulation as well as ketotifen, a known mast cells stabilizer. These results confirm the described data that the pentacyclic triterpenes are able to suppress experimental pruritus on scratching behavior induced by compound 48/80 or dextran T40 in mice and this effect be related to a modulatory action in the mast cell activation (Oliveira et al., 2004).

Thus, we may consider that the antipruritic effect of LPEE, LPEF and MT is due to its mast cells membrane stabilization property. Furthermore *Lecythis pisonis* could inhibit the paw swelling response, induced by carrageenan in rats. The model of carrageenan-induced paw edema is widely used for the evaluation of new compounds with anti-inflammatory potential, which has frequently been used to assess the anti-edematous effect of natural products. Carrageenan is a sulfated polysaccharide derived from seaweed used as inflammatory stimulus. Its administration causes a swelling with an initial rapid phase and a prolonged late phase. The first phase (1–2 h after the phlogistic agent) is mediated by histamine release, serotonin and bradykinin, whereas the second phase (3–4 h) is mainly related to the increased prostaglandins synthesis in the tissues (Winter et al., 1962; Kale et al., 2007).

The LPEE and LPEF significantly inhibited the paw edema induced by carrageenin. The LPEE reduced the edema effectively until the third hour (Table 4). However, the anti-edematogenic effect of LPEF remained at all evaluation times (Table 5). This anti-inflammatory response was also significant in rats pre-treated with indomethacin, a known cyclooxygenase inhibitor. These data suggest that the antiedematogenic activity of the LPEE and LPFE is related to the early inflammatory mediators released during the edema formation. Among these mediators, histamine is highlighted for being responsible for vasodilation and plasma extravasation, which occurs in the first phase of carrageenan-induced edema in rats (Di Rosa et al., 1971). Taken together, these observations suggest *Lecythis pisonis* that suppress the function of mast cell secretogogues.

It is known that drugs with sedative activity can also inhibit scratching behavior (Watanable et al., 1999). However, the doses used in experiments, LPEE did not alter the locomotor activity of

Table 5
Antiedematogenic effect of the ethereal fraction of *Lecythis pisonis* leaves (LPEF) in the carrageenan-induced hind paw edema.

Treatment (mg/kg, p.o.)	Paw diameter (mm)					
	1 h	2 h	3 h	4 h	5 h	6 h
Vehicle	1.434 ± 0.098	2.236 ± 0.092	2.718 ± 0.157	2.097 ± 0.144	1.865 ± 0.209	1.190 ± 0.219
LPEF (50)	1.904 ± 0.094	2.320 ± 0.112	3.101 ± 0.139	2.523 ± 0.102	1.856 ± 0.161	1.422 ± 0.131
LPEF (100)	1.187 ± 0.190**	1.779 ± 0.255**	2.274 ± 0.157*	1.994 ± 0.170	1.926 ± 0.235	1.325 ± 0.139
LPEF (200)	1.192 ± 0.070**	2.052 ± 0.182*	2.250 ± 0.067**	2.050 ± 0.112	1.698 ± 0.125	1.537 ± 0.070
Indomethacin (10)	0.214 ± 0.051***	0.634 ± 0.127***	0.243 ± 0.109***	0.379 ± 0.140***	0.475 ± 0.136***	0.280 ± 0.097***

Rats were treated with LPEF 60 min (p.o.) before carrageenan-induced hind paw edema. Data represent the mean ± S.E.M. of 5–8 animals. The symbols report significance level.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

animals, suggesting that the sedative action of LPEE may not be involved in the inhibitory mechanism of scratching behavior.

It possible to find many studies that show the efficacy of several natural compounds classes originated from vegetable species such as flavonols, naphthoquinones, saponins and triterpenes, related with dependent and-independent histamine pruritus (Ishiguro and Oku, 1994; Oliveira et al., 2004; Fu et al., 2005). The major metabolites of the ethanolic extract and active ethereal fraction were isolated and identified as a binary mixture of pentacyclic triterpenes: ursolic and oleanolic acids. This mixture was isolated in amounts of 4.75% (475 mg) in the ethereal fraction (LPEF) and 0.57% (1.14 g) in the crude extract (ethanolic extract, LPEE). These findings are consistent with the literature, as Shin et al. (2005) show antipruritic activity of triterpene saponin, gisenosideo Rb1, isolated from ginseng on scratching behavior animal model induced by compound 48/80 in ICR mice. Regarding the important yield of these compounds, and based on literature it can be suggested that these triterpenes (mixture of ursolic and oleanolic acids) could be responsible for the activity observed in this study.

In conclusion, the results obtained in this study provide, for the first time, experimental evidence that justifies the use of popular *Lecythis pisonis* leaves in the treatment of pruritus. These data clearly show the therapeutic potential of this plant and its compounds for the development of new substances with antipruritic activities.

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