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Anti-inflammatory, analgesic and anti-oedematous effects of *Lafoensia pacari* extract and ellagic acid

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Abstract

Lafoensia pacari St. Hil. (Lythraceae) is used in traditional medicine to treat inflammation. Previously, we demonstrated the anti-inflammatory effect that the ethanolic extract of *L. pacari* has in *Toxocara canis* infection (a model of systemic eosinophilia). In this study, we tested the anti-inflammatory activity of the same *L. pacari* extract in mice injected intraperitoneally with β-glucan present in fraction 1 (F1) of the *Histoplasma capsulatum* cell wall (a model of acute eosinophilic inflammation). We also determined the anti-oedematous, analgesic and anti-pyretic effects of *L. pacari* extract in carrageenan-induced paw oedema, acetic acid writhing and LPS-induced fever, respectively. *L. pacari* extract significantly inhibited leucocyte recruitment into the peritoneal cavity induced by β-glucan. In addition, the *L. pacari* extract presented significant analgesic, anti-oedematous and anti-pyretic effects. Bioassay-guided fractionation of the *L. pacari* extract in the F1 model led us to identify ellagic acid. As did the extract, ellagic acid presented anti-inflammatory, anti-oedematous and analgesic effects. However, ellagic acid had no anti-pyretic effect, suggesting that other compounds present in the plant stem are responsible for this effect. Nevertheless, our results demonstrate potential therapeutic effects of *L. pacari* extract and ellagic acid, providing new prospects for the development of drugs to treat pain, oedema and inflammation.

Introduction

It is widely known that various plants present significant anti-inflammatory properties. The Lythraceae family includes two species that possess anti-inflammatory, analgesic and anti-pyretic properties: *Lawsonia inermis* and *Heimia salicifolia*. Lawsone and cryogenine, compounds isolated from *L. inermis* and *H. salicifolia*, respectively, have been found to have anti-inflammatory, analgesic and anti-pyretic effects in several experimental models (Kaplan et al 1967; Watson & Malone 1977; Ali et al 1995). *Lafoensia pacari* St. Hil. (Lythraceae), known in the state of Mato Grosso (Brazil) as mangava brava or dedaleira, has been used in traditional medicine to treat gastric ulcers and inflammation (Albuquerque et al 1996; Solon et al 2000). Albuquerque et al (1996) demonstrated that the ethanolic extract of *L. pacari* stem bark has an anti-inflammatory effect in carrageenan-induced peritonitis in mice. In a previous study, we found that treatment with an aqueous suspension of the ethanolic extract of *L. pacari* resulted in significant modulation of eosinophil and mononuclear cell migration, as well as of IL-5 production in the blood, bone marrow, peritoneal cavity and bronchoalveolar lavage fluid, in mice infected with *Toxocara canis* (Rogerio et al 2003). In peritonitis induced by β-glucan, or fraction 1 (F1), present in the cell wall of *Histoplasma capsulatum* (a model of acute eosinophilic inflammation) (Medeiros et al 1999), bioassay-guided fractionation of ethanolic extract from *L pacari* (Medeiros et al 1999) led us to identify ellagic acid as the compound responsible for the anti-inflammatory effect. Ellagic acid possesses a variety of biological properties, exhibiting anti-oxidant (Suzuki et al 1990; Solon et al 2000; Priyadarsini et al 2002), anti-fibrosis (Thresiamma & Kuttan 1996), anti-atherogenic (Anderson et al 2001) and anti-cancer (Mukhtar et al 1986; Narayanan et al 1999) effects.

In this study, the anti-nociceptive, anti-oedematous, anti-pyretic and anti-inflammatory effects of the *L. pacari* ethanolic extract and of ellagic acid were investigated.

Materials and Methods

Ethanolic extracts

L. pacari stem bark was collected in June of 1998 in the city of Cuiabá, in the state of Mato Grosso, Brazil. A voucher specimen (No. 25282) was deposited at the Central Herbarium of the Universidade Federal do Mato Grosso. The crude ethanolic extract was prepared as described previously (Rogerio et al 2003).

Fractionation of the ethanolic extract

The *L. pacari* ethanolic extract (29 g) was dissolved in EtOH–H_2O (7:3) and partitioned successively with hexane and dichloromethane to produce a hexane extract (0.59 g, designated fraction A), a dichloromethane extract (1.27 g, fraction B) and an EtOH–H_2O extract (22 g, fraction C). A portion of fraction C (14 g) was fractionated on a reversed-phase C18 column (100 g) using MeOH–H_2O (H_2O, MeOH–H_2O 1:1, and MeOH) to produce the fractions C.1 (3.64 g), C.2 (2.48 g) and C.3 (0.074 g). Fraction C.2 (2.48 g) was fractionated on a reversed-phase C18 column (100 g). We used MeOH–H_2O (1:3, 7:13, 4:6, 9:11, 1:1) and finally MeOH to produce fractions C.2.1 (1.28 g), C.2.2 (0.18 g), C.2.3 (0.06 g), C.2.4 (0.04 g), C.2.5 (0.036 g) and C.2.6 (0.015). Part of the C.2.2 fraction (0.15 g) was chromatographed on a Sephadex LH-20 column (5×150 mm, MeOH) to produce 50 more fractions of 10 mL each. All fractions were then analysed using HPLC. Fractions 10–15 were reunited (fraction R, 7.5 mg), and HPLC was used to analyse the product of that union in comparison with ellagic acid, which was purchased from Alfa Aesar Organics (Ward Hill, MA).

Chromatographic separation and identification of ellagic acid

The fractions detailed above were further analysed using a reversed-phase HPLC system (Shimadzu, Kyoto, Japan) consisting of two SPD-M10A solvent pumps, an SCL-10A system controller, a LC-10A column oven and a SIL-10AD auto-injector with a 20-μL loop. Separations were carried out at 25°C on a Supelconsil LC-18 column (250 mm×4.6 mm, 5 μm; Supelco, Bellefonte, PA) and a Supelcosil LC-18 pre-column (4.0 mm×4.0 mm, 5 μm; Supelco). Elution was carried out at a flow-rate of 1.0 mL min^{-1} using 1% acetic acid as the mobile phase in both H_2O (solvent A) and MeOH (solvent B). The following elution gradients were used: 0.0 min, 0% B; 32 min, 100% B; 35 min, 0% B.

Elucidation of the ellagic acid structure

The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance DRX 400 spectrometer (Bruker Biospin, Billerica, MA). Identification of this compound was made by comparing ^{1}H NMR and ^{13}C NMR data with those reported previously (Li et al 1999).

Animals

Female BALB/c mice, 15–20 g, were used to evaluated leucocyte recruitment; female Swiss mice, 15–20 g, were used in oedema and writhing tests; and male Wistar rats, 180–200 g, were used in fever determination. All animals were obtained from the animal facilities of the Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, and were maintained under standard laboratory conditions (21–24°C at 40–60% relative humidity on a 12-h light–dark cycle). All experiments were approved by, and conducted in accordance with the guidelines established by, the animal care committee of the local institution (protocol No. 04.1.659.53.5).

Preparation and fractionation of cell walls

The procedure used was essentially the same as that described previously (Silva & Fazioli 1985; Medeiros et al 1999). The resulting white powder was designated F1 of the dead yeast form of *H. capsulatum*.

Peritonitis induced by F1 and treatments

Leucocyte recruitment into the peritoneal cavity was induced in BALB/c mice by inoculation of 100 μg of F1 in 1 mL of phosphate-buffered saline (PBS). Control group mice were injected with the same volume of PBS (Medeiros et al 1999). The mice were treated with water, *L. pacari* extract or fractions, perorally by gavage, at 1 h before intraperitoneal injection of the stimulus and again at 1 h before sacrifice, which was achieved through administration of an overdose of sodium pentobarbital at 24 h after intraperitoneal injection. Estimated doses in bioassay-guided fractionation of *L. pacari* extract were based on dose of the ethanolic extract (200 mg kg^{-1}). A group of mice treated with dexamethasone (1 mg kg^{-1}, s.c.) was used for comparison. The total and differential cell counts were performed as described previously (Faccioli et al 1990).

Mouse writhing test

This test was based on the method devised by Koster et al (1959). Writhing was induced by intraperitoneal administration of 0.2 mL of 0.6% acetic acid (v/v) in Swiss mice. At 30 min before intraperitoneal administration, the mice received one of the following (p.o.): water; Tris; indometacin (10 mg kg^{-1}) diluted in Tris (pH 8.2); *L. pacari* extract (8, 40, or 200 mg kg^{-1}) suspended in water; or ellagic acid (0.1, 1, 10 or 100 mg kg^{-1}) suspended in water. The writhing was observed over a 15-min period beginning immediately after intraperitoneal injection.

Paw oedema

Paw oedema was induced in a single hind paw of each mouse by intraplantar injection of 50 μL of 1% (w/v) lambda carrageenan (Levy 1969). The contralateral paw was injected with the same volume of PBS and served as a control. After injection, the difference in footpad thickness between the right and left paw was measured with a pair of dial thickness gauge callipers

(Mitutoyo, Kawasaki, Japan). Paw oedema was measured at 1, 2, 3 and 4 h. Mice received, (p.o.), *L. pacari* extract (8, 40 or 200 mg kg^{-1}), ellagic acid (0.1, 1 or 10 mg kg^{-1}), or indometacin (10 mg kg^{-1}) at 30 min before carrageenan administration.

Anti-pyretic effects in rats

The procedure used was the same as that described previously (Fabrício et al 2005). At 30 min before the injection of LPS (5 μg kg^{-1}, i.v.), rats received (p.o.) *L. pacari* extract (8, 40 or 200 mg kg^{-1}), ellagic acid (0.1, 1 or 10 mg kg^{-1}) or indometacin (10 mg kg^{-1}). Rats in one group were injected with saline only and used as controls. All injections were given between 1000 and 1100 h to avoid circadian variations in responsiveness in a temperature-controlled room (28 ± 1°C).

Statistical analysis

The data are reported as means ± s.e.m. and are representative of two or three different experiments. The means from different treatments in each individual experiment were compared by analysis of variance. When significant differences were identified, individual comparisons were subsequently made with Bonferroni's *t*-test for unpaired values. Two-way analysis of variance, followed by the Bonferroni test, was used to calculate the significance of the differences among the temperatures. $P < 0.05$ was considered statistically significant.

Results

Bioassay-guided fractionation in the F1-induced peritonitis model

At 24 h after intraperitoneal injection of F1, the numbers of neutrophils, eosinophils and mononuclear cells in the peritoneal cavities of mice receiving F1 were significantly higher than those observed in the peritoneal cavities of mice receiving PBS alone (Figure 1). This finding is in agreement with those of previous studies (Medeiros et al 1999). In this model, bioassay-guided fractionation procedures were conducted using the ethanolic extract of *L. pacari*. Each partition or fraction was then administered to mice in doses estimated according to their ratio in ethanolic extract. The ethanolic extract was first partitioned to produce fractions A, B and C. In the peritoneal cavities of mice receiving ethanolic extract of *L. pacari* (200 mg kg^{-1}) or fraction C (153 mg kg^{-1}), lower numbers of neutrophils (34% and 30% lower, respectively) and eosinophils (96% and 63% lower, respectively) were observed in comparison with those seen in the peritoneal cavities of mice injected with F1 and treated only with water (p.o.). Treatment of mice with fraction A (4 mg kg^{-1}) decreased the numbers of eosinophils by only 66%. However, mice treated with fraction B (10 mg kg^{-1}) presented significant increases in mononuclear cell numbers (Figure 1). Fraction C exhibited potent anti-inflammatory properties and was therefore fractionated on

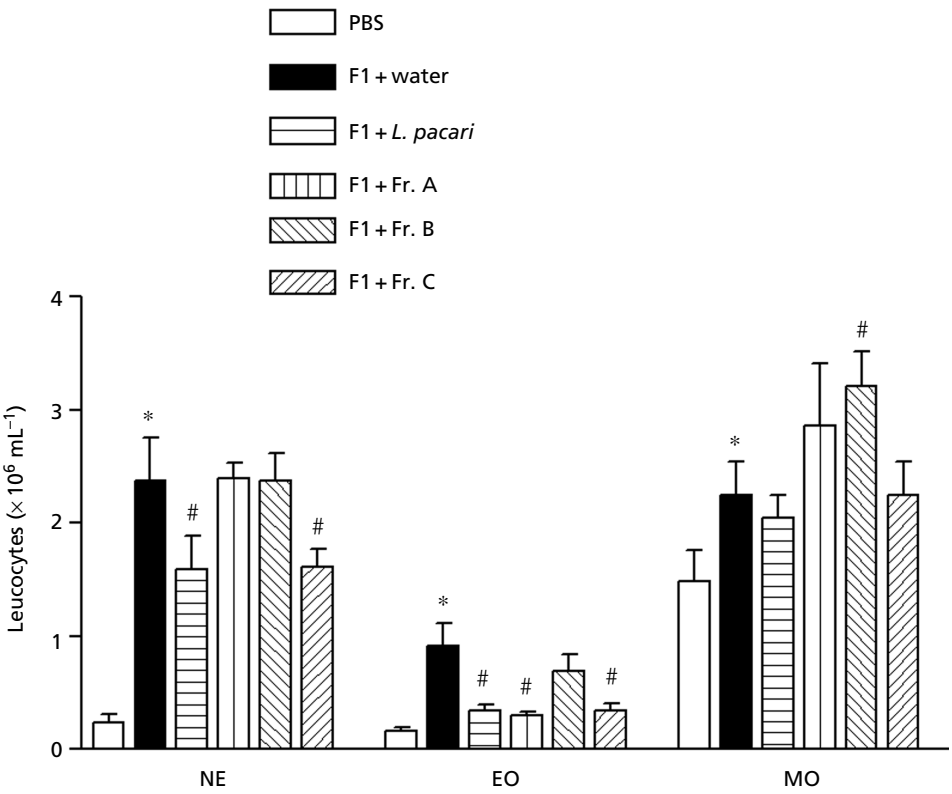

Figure 1 Effect of *L. pacari* extract (200 mg kg^{-1}), as well as of fractions A (4 mg kg^{-1}), B (10 mg kg^{-1}), and C (153 mg kg^{-1}), on the recruitment of neutrophils (NE), eosinophils (EO) and mononuclear cells (MO) induced by intraperitoneal inoculation of mice with 100 μg of F1 isolated from *H. capsulatum* cell walls. Cell counts are expressed as the mean ± s.e.m. for the mice tested (n = 14). *$P < 0.05$ when compared with the control (PBS) group; #$P < 0.05$ when compared with the F1 + water group.

a reversed-phase C18 column, yielding the fractions C.1, C.2 and C.3. Fraction C (153 mg kg^{-1}) was used as a positive anti-inflammatory control. Mice treated with fraction C.1 (44 mg kg^{-1}) or fraction C.2 (30 mg kg^{-1}) presented significantly lower numbers of neutrophils (24% and 26% lower, respectively) and eosinophils (26% and 42% lower, respectively) than did mice injected with F1 and treated with water (Figure 2). In mice treated with fraction C.3 (1 mg kg^{-1}), eosinophil numbers were significantly (80%) lower, although mononuclear cell numbers were higher, than in mice injected with F1 and treated with vehicle alone (Figure 2).

The C.2 and C.3 fractions presented potent anti-inflammatory effects. However, due to its greater mass, fraction C.2 was chosen for fractionation on a reversed-phase C18 column, resulting in fractions C.2.1, C.2.2, C.2.3, C.2.4, C.2.5 and C.2.6. We used fraction C.2 (30 mg kg^{-1}) as a positive control for comparison purposes. Fraction C.2.2 (15 mg kg^{-1}) decreased neutrophil and eosinophil numbers significantly (by 62% and 75%, respectively) in comparison with those observed in mice injected with F1 and treated only with water. Fractions C.2.3 (4.8 mg kg^{-1}), C.2.4 (3.4 mg kg^{-1}), C.2.5 (2.9 mg kg^{-1}) and C.2.6 (7.4 mg kg^{-1}) induced significant (57%, 66%, 65% and 69%, respectively) decreases in eosinophil numbers. In addition, fractions C.2.2 and C.2.3 decreased mononuclear cell numbers significantly (by 33% and 30%, respectively) when compared with those seen in mice injected with fraction F1 and treated with vehicle alone (Figure 3). No significant alterations were observed in mice treated with fraction C.2.1.

Fraction C.2.2 exhibited a potent anti-inflammatory effect and was chromatographed on a Sephadex LH-20 column, yielding an additional 50 fractions. Fractions 10–15 were united (into fraction R). Ellagic acid was identified by analytical HPLC and confirmed by ^{1}H and ^{13}C NMR spectra. Due to the small amount of ellagic acid mass obtained (7.5 mg), we chose to carry out the subsequent experiments using standard ellagic acid. The effects of ellagic acid on F1-induced peritonitis were thus analysed. In mice treated with *L. pacari* extract, dexamethasone (1 mg kg^{-1}), or ellagic acid (10 mg kg^{-1}), neutrophil and eosinophil numbers were lower (46%, 61% and 65%, respectively; and 72%, 57% and 74%, respectively) than those seen in mice injected with F1 and treated with vehicle alone (Figure 4). In addition, dexamethasone administration (1 mg kg^{-1}) decreased mononuclear cell counts significantly (by 33%). In Figure 4, we also illustrate the profiles of cells recovered from the peritoneal cavities of F1-inoculated mice treated with water, *L. pacari*, ellagic acid or dexamethasone. The mice treated with *L. pacari*, ellagic acid or dexamethasone presented less inflammation, as shown by the lower numbers of neutrophils and eosinophils.

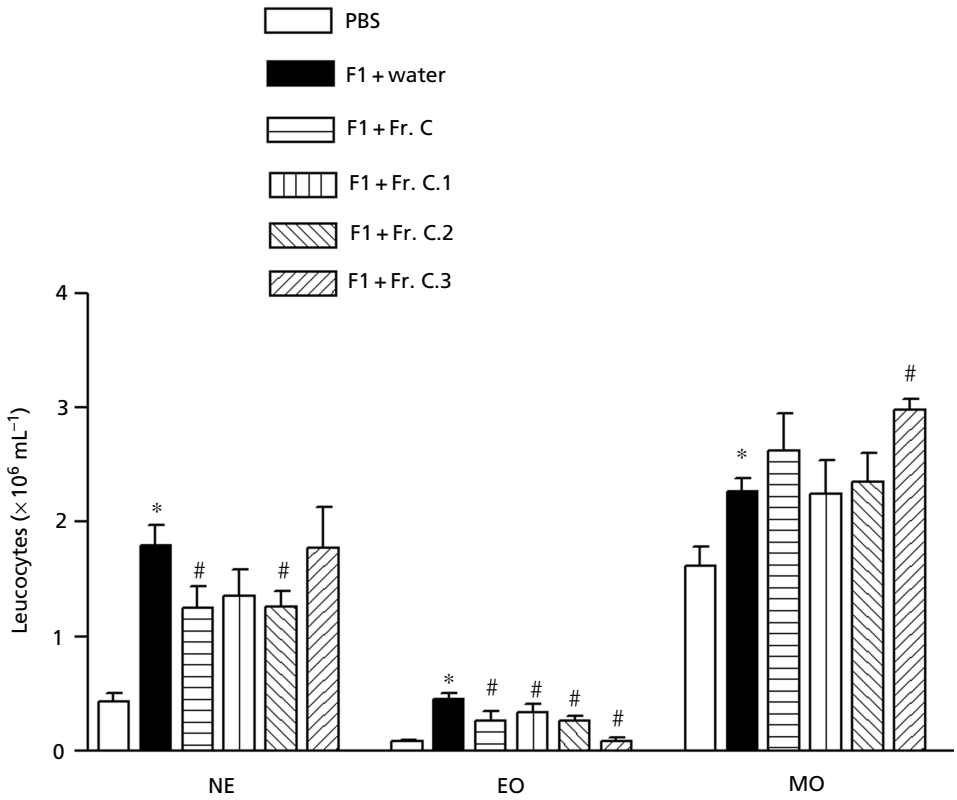

Figure 2 Effect of fraction (Fr.) C (153 mg kg^{-1}), Fr. C.1 (44 mg kg^{-1}), Fr. C.2 (30 mg kg^{-1}) and Fr. C.3 (1 mg kg^{-1}) on the neutrophil (NE), eosinophil (EO) and mononuclear cell (MO) recruitment induced by intraperitoneal inoculation of mice with 100 μg of F1 isolated from *H. capsulatum* cell walls. Cell numbers are expressed as the mean ± s.e.m. for the mice tested (n = 14). *$P < 0.05$ when compared with the control (PBS) group; #$P < 0.05$ when compared with the F1 + water group.

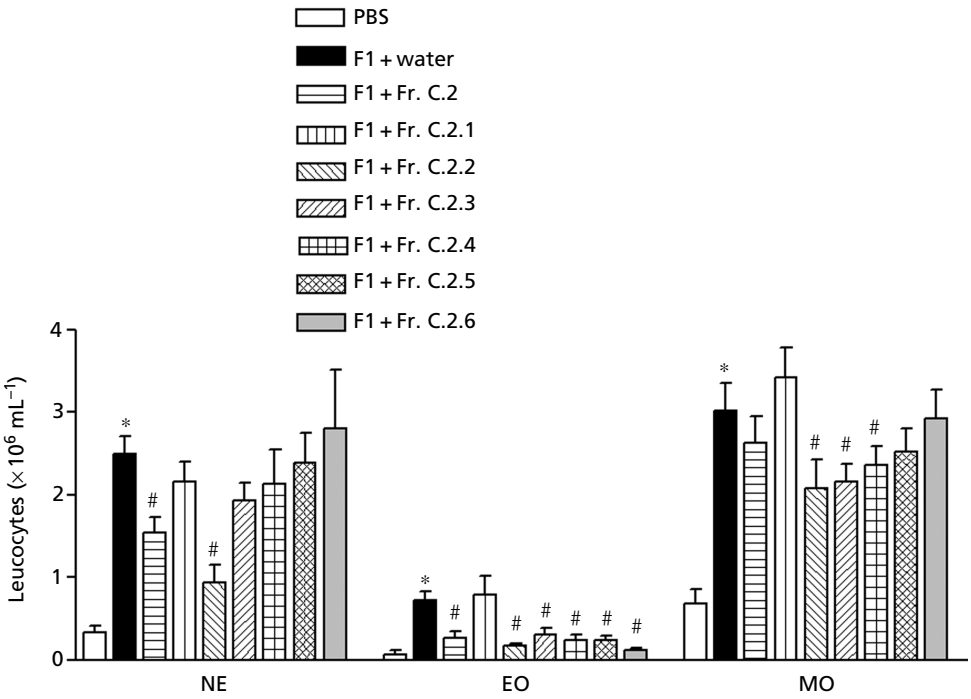

Figure 3 Effect of fractions (Fr.) C.2 (30 mg kg^{-1}), C.2.1 (103 mg kg^{-1}), C.2.2 (15 mg kg^{-1}), C.2.3 (4.8 mg kg^{-1}), C.2.4 (3.4 mg kg^{-1}), C.2.5 (2.9 mg kg^{-1}) and C.2.6 (7.4 mg kg^{-1}) on the neutrophil (NE), eosinophil (EO) and mononuclear cell (MO) recruitment induced by intraperitoneal inoculation of mice with 100 μg of F1 isolated from *H. capsulatum* cell walls. Cell numbers are expressed as the mean ± s.e.m. for the mice tested (n = 14). *$P < 0.05$ when compared with the control (PBS) group; #$P < 0.05$ when compared with the F1 + water group.

Mouse writhing test

Figure 5A, B shows the number of acetic acid-induced abdominal contractions in mice treated with vehicle (water or Tris). Administration of *L. pacari* extract at doses of 40 mg kg^{-1} or 200 mg kg^{-1} inhibited such contractions significantly (by 43% in both cases) in comparison with the number of contractions observed in mice treated with vehicle only. Administration of indometacin (10 mg kg^{-1}, p.o.) resulted in even greater (94%) inhibition of the contractions (Figure 5A).

The number of acetic acid-induced abdominal contractions in mice treated with ellagic acid at doses of 0.1, 1, 10, or 100 mg kg^{-1} were lower by 25%, 65%, 65% and 60%, respectively, than in control mice treated with vehicle only (Figure 5B).

Paw oedema

Intraplantar injection of carrageenan induced significantly greater mouse paw volume than that seen in the contralateral paw injected with PBS. Mice treated with *L. pacari* extract (200 mg kg^{-1}), ellagic acid (0.1 and 100 mg kg^{-1}) or indometacin (10 mg kg^{-1}) presented significantly less pronounced oedematous responses to carrageenan, beginning at 1 h after carrageenan administration and continuing throughout the experimental period (Figure 6A, B). Mice treated with ellagic acid (1 and 10 mg kg^{-1}) presented oedema inhibition only at 1, 2 and 3 h after carrageenan administration (Figure 6B).

Anti-pyretic properties

Figure 7A shows that indometacin (10 mg kg^{-1}) and *L. pacari* extract (200 mg kg^{-1}) had significant anti-pyretic effects on LPS-induced fever in rats, which was, however, unaffected by administration of ellagic acid (0.1, 1, 10 or 100 mg kg^{-1}) (Figure 7B).

Discussion

The genus *Lafoensia* is a member of the family Lythraceae, which are found throughout tropical and subtropical regions of the world. Lythraceae reportedly contain acids, triterpenes, saponins, quinines, alkaloids, flavonoids, phenols, tannins, chalcones, aurones, leucoanthocyanidins, anthraquinones, saponins and acetophenones (Garcez et al 1998; de Carvalho et al 1999; Solon et al 2000; de Lima et al 2006). Ellagic acid, a tannin, has been identified in stem bark extract (Solon et al 2000), and the stem bark of the plant is used in Brazil as an anti-inflammatory agent.

Through bioassay-guided fractionation of *L. pacari* extract in a model of acute eosinophilic inflammation (Medeiros et al 1999), we isolated and chemically characterised ellagic acid (Figures 1, 2 and 3). We found ellagic acid to have a potent anti-inflammatory effect, inhibiting F1 inoculation-induced recruitment of neutrophils and eosinophils into the peritoneal cavity (Figure 4). These results are in agreement with those of Albuquerque et al (1996), who demonstrated that *L. pacari*

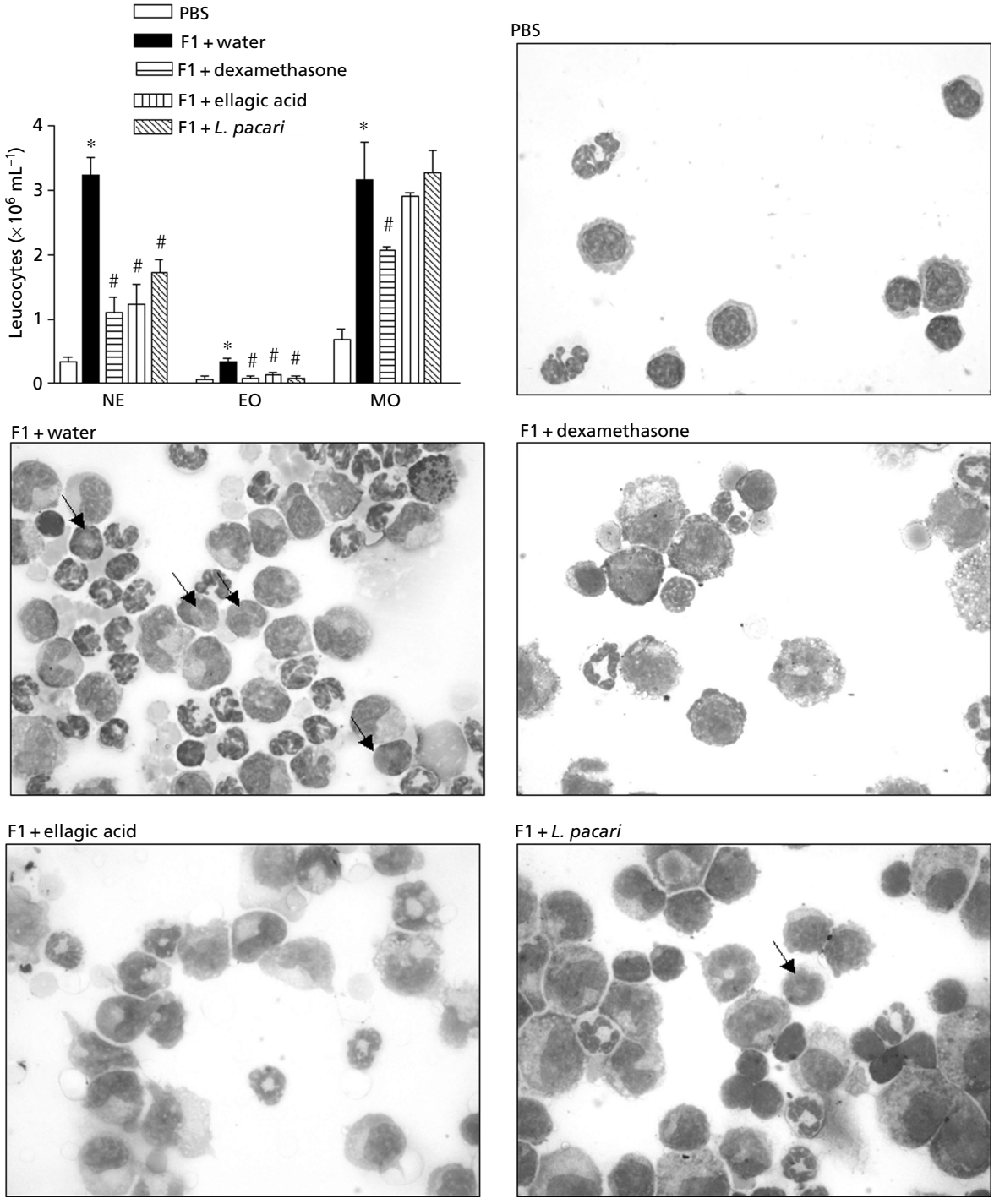

Figure 4 Effect of ellagic acid (10 mg kg^{-1}), *L. pacari* extract (200 mg kg^{-1}) and dexamethasone (1 mg kg^{-1}) on the neutrophil (NE), eosinophil (EO) and mononuclear cell (MO) recruitment induced by intraperitoneal inoculation of mice with 100 μg of F1 isolated from *H. capsulatum* cell walls. Cell numbers are expressed as the mean ± s.e.m. for the mice tested (n = 14). *$P < 0.05$ when compared with the control (PBS) group; $^{\#}P < 0.05$ when compared with the F1 + water group. Photomicrographs show cells present in the peritoneal cavity of mice inoculated with PBS or F1 and treated with water, dexamethasone, ellagic acid or *L. pacari*. Arrows indicate eosinophils in peritoneal exudate. Magnification, × 1000.

extract minimised carrageenan-induced neutrophil recruitment into the peritoneal cavities of mice. These findings are also in agreement with those of previous findings conducted by our group, in which we demonstrated the anti-eosinophilic effect of *L. pacari* extract on toxocariasis (Rogerio et al 2003). This suggests that ellagic acid is the secondary metabolite responsible for the eosinophilic activity seen in this model. To the best of our knowledge, this is the first study

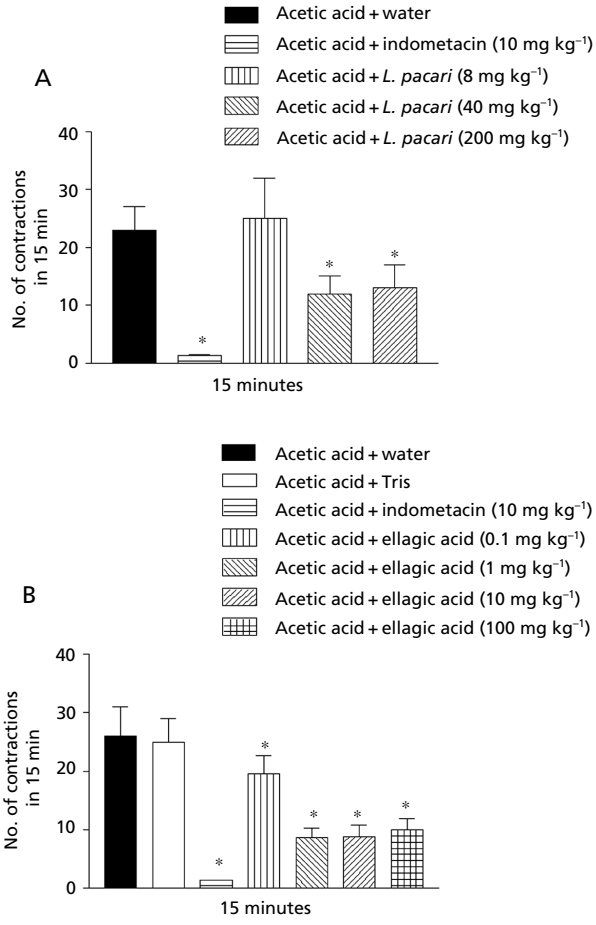

Figure 5 Analgesic effect of the *L. pacari* extract (A) and ellagic acid (B) in the acetic acid-induced mouse writhing test. At 30 min before intraperitoneal injection of 0.6% acetic acid (0.2 mL), the mice received (p.o.) 0.3 mL of water, 0.3 mL of Tris, 10 mg kg^{-1} of indometacin, *L. pacari* extract at the doses 8, 40 or 200 mg kg^{-1}, or ellagic acid at the doses 0.1, 1, 10, or 100 mg kg^{-1}. Data represent the number of contractions observed over a 15-min period in the mice tested (n = 14). $^{*}P < 0.05$ compared with mice receiving water only.

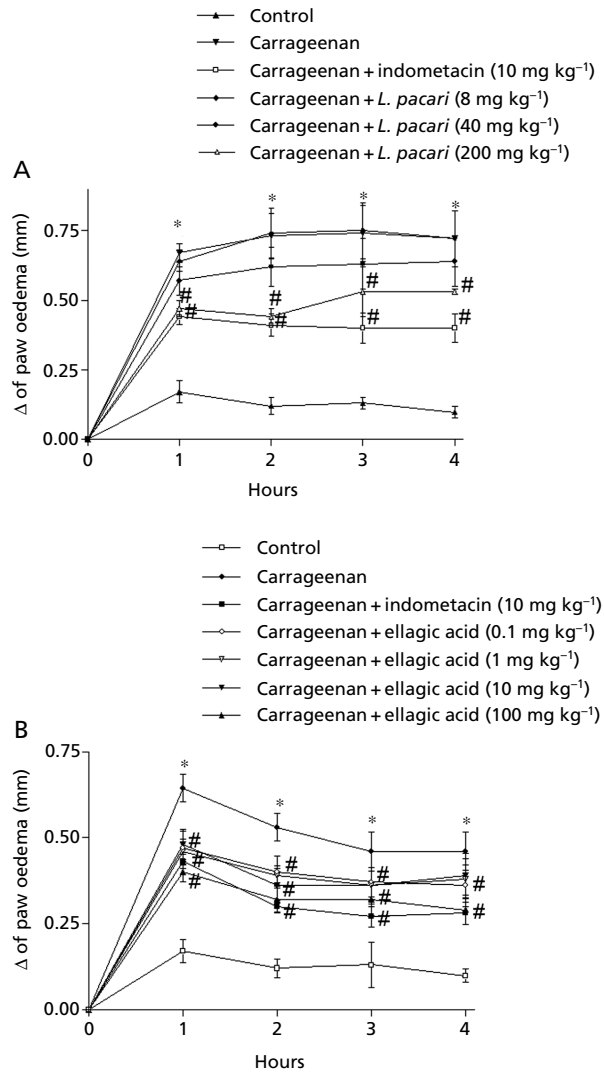

Figure 6 Anti-oedematous effects of *L. pacari* extract (A) and ellagic acid (B) on carrageenan-induced mouse paw oedema. Mice received *L. pacari* extract (8, 40 or 200 mg kg^{-1}), ellagic acid (0.1, 1, 10 or 100 mg kg^{-1}), indometacin (10 mg kg^{-1}) or vehicle (water, 0.3 mL, p.o.) at 30 min before intraplantar injection of 1% carrageenan (50 μL). The values represent the mean ± s.e.m. of the variation in the paw volume for 10 mice per group. $^{*}P < 0.05$, indometacin versus vehicle; $^{\#}P < 0.05$, *L. pacari* extract or ellagic acid versus vehicle.

demonstrating the capacity of ellagic acid to inhibit cell recruitment.

Ellagic acid exhibits a wide range of biological properties, exerting anti-oxidant (Priyadarsini et al 2002), anti-fibrosis (Thresiamma & Kuttan 1996) and anti-cancer (Narayanan et al 1999) effects. It has been shown that ellagic acid protects against gastric injury induced by ischaemia/reperfusion (Iino et al 2002), as well as against carbon tetrachloride-induced liver fibrosis (Thresiamma & Kuttan 1996). Damas & Volon (1979) demonstrated an anti-inflammatory effect of ellagic acid in rat paw oedema induced by the compound 48/80 and by carrageenan. These results are in agreement with our findings that *L. pacari* extract and ellagic acid inhibited carrageenan-induced paw oedema (Figure 6). In addition, *L. pacari* extract and ellagic acid inhibited the number of acetic acid-induced abdominal contractions in mice (Figure 5). However, only *L. pacari* extract inhibited LPS-induced fever in rats (Figure 7).

Tannins are water-soluble polyphenols that are found in food grains, as well as in fruits, and are widely distributed throughout the plant kingdom. The mechanisms underlying the anti-inflammatory effect of tannins include the scavenging of radicals (anti-oxidant effect) (Hagerman et al 1999) and inhibition of cytokines (Feldman et al 2001), inducible nitric oxide synthase and cyclooxygenase-2 inhibition (Lee et al 2003). Also, ellagic acid has been found to inhibit the production of MCP-1 induced by IL-1β and TNF-α through the inhibition of MAPK activation (Masamune et al 2005), the TPA-induced phosphorylation of ERK1/2, p38, and JNK1/2 (Yoshimura et al 2005), activation of NF-κB and IKKα, and phosphorylation and degradation of IκBα. (Afaq et al 2003). Both NF-κB and AP-1 are considered redox-sensitive transcription factors (Adcock et al 1994; Winyard & Blake 1997; Krishna et al 1998; Haddad et al 2000). The anti-oxidant

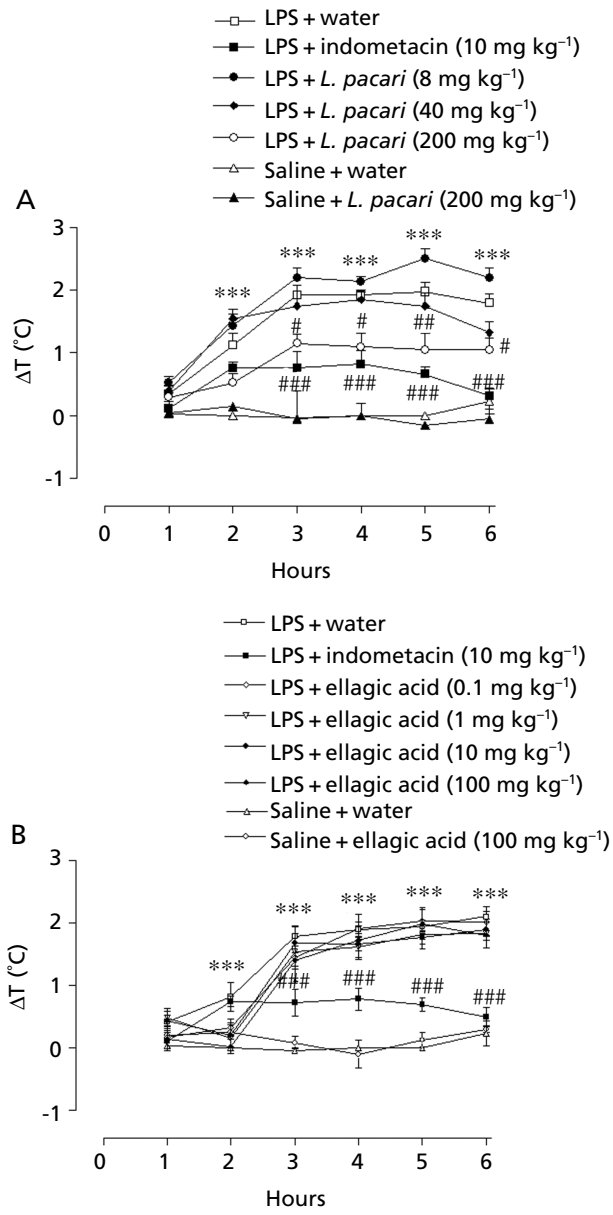

Figure 7 Anti-pyretic effect of *L. pacari* (A) or ellagic acid (B) on fever induced by administration of LPS (5 μg kg^{-1}). Rats were treated p.o. with water, *L. pacari* (8, 40 or 200 mg kg^{-1}), ellagic acid (0.1, 1, 10 or 100 mg kg^{-1}) or indometacin (10 mg kg^{-1}) at 30 min before intravenous injection of LPS or saline. Values represent the means ± s.e.m. of the changes in rectal temperature (°C) of 14 rats. Basal rectal temperatures ranged from 36.8 to 37.4°C. Using two-way analysis of variance, we observed significant differences for the main effect of treatment and treatment–time interaction ($P < 0.0001$). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared with the saline-treated control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ when compared with the LPS + water group

effect of ellagic acid may modulate the activation these transcription factors (Erdelyi et al 2005). Therefore, it is possible that the anti-oedematous and analgesic effects, as well as the inhibitory effects on cell migration, that *L. pacari* extract and ellagic acid have in mice are related to inhibition of the synthesis of common mediators involved in these responses. The anti-pyretic effects of *L. pacari* extracts cannot be attributed to the action of ellagic acid alone, suggesting that the plant stem contains other anti-pyretic compounds. However, the fact that fever was unaffected by the administration of ellagic acid is interesting since fever has positive effects (e.g. decreased bacterial growth and stimulation of host defence mechanisms) (Vaughn et al 1980).

Conclusions

Our findings provide new perspectives on the therapeutic use of *L. pacari* extract and ellagic acid as analgesic, anti-oedematous and anti-inflammatory agents, especially for the treatment of allergic diseases.

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