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Research Paper

Antiulcerogenic and anti-inflammatory activities of the essential oil from *Pterodon emarginatus* seeds

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Abstract

Objectives The objective of this work was to investigate the antiulcerogenic and anti-inflammatory activities of the essential oil from *Pterodon emarginatus* seeds.
Methods The following tests were used: ulcers induced by ethanol, indometacin and HCl/ethanol, and pleurisy induced by carrageenan in Swiss albino rats. The rats were treated by the oral route with essential oil of *P. emarginatus* seeds.
Key findings The essential oil at 100, 300 and 500 mg/kg exhibited significant protection against ulcers induced by ethanol, indometacin and HCl/ethanol ($P < 0.001$). The essential oil caused a marked reduction in the exudate volume and inhibited leucocyte and neutrophil influx ($P < 0.05$) in carrageenan-induced pleurisy. Moreover, the essential oil significantly decreased nitric oxide (NO) and interleukin-1 (IL-1) levels, without affecting tumour necrosis factor-α production.
Conclusions The results demonstrated the marked antiulcerogenic and anti-inflammatory effects of the essential oil from *P. emarginatus,* which are, at least in part, a consequence of NO and IL-1 modulation. *P. emarginatus* or its constituents might represent new therapeutic options to treat gastric ulcers and inflammatory diseases.
Keywords anti-inflammatory; antiulcerogenic; essential oil; *P. emarginatus*

Introduction

Since ancient times, people have been relying on plants in either prophylactic or the therapeutic arsenal to restore and maintain health, and plants are well known as an important source of many biologically active compounds. A study reported that there has been a growing interest in plants as a significant source of new drugs.[1]

Pterodon emarginatus Vogel is a plant from the Brazilian cerrado belonging to the Leguminosae family. This family includes several species that have been widely used in folk medicine due to their antiulcerogenic, analgesic and anti-inflammatory activities, among others. *P. emarginatus* is commonly known as 'sucupira branca' or 'faveiro' and it is used in Brazilian folk medicine to treat rheumatism, sore throats and respiratory dysfunction (bronchitis and amigdalitis) in addition to possessing anti-inflammatory, analgesic, depurative and tonic activity. Its seeds are commercially available at medicinal flora markets, being largely used because of their pharmacological properties.[2,3] In popular medicine, seeds of *P. emarginatus* are ground in hydroalcoholic solution using 50 g of seeds crushed in 250 ml of diluents (concentration of 200 mg/ml) of ethanol or alcoholic drink, such as brandy, under static maceration for 24–48 h at room temperature. After preparation, the extract is orally consumed at a dose of 40 ml/day, divided into two doses, for 7 days.

The genus *Pterodon* comprises five Brazilian native species: *P. abruptus* Benth, *P. appariciori* Pedersoli, *P. emarginatus* Vogel, *P. pubescens* Benth and *P. polygalaeflorus* Benth. Chemical investigation of these species was initiated by the discovery that the fruit oil of *P. pubescens* inhibited the penetration of schistosome cercaria in the skin, a property that was attributed to 14,15-epoxygeranylgeraniol and later to the accompanying linear diterpenoid 14,15-dihydroxy-14,15-dihydrogeranylgeraniol. Geranylgeraniol itself also occurs in *P. pubescens*, and as the characteristic floral odour of this diterpene and similar biological activity are observed for the fruit oils of *P. emarginatus*, *P. polygalaeflorus* and *P. apparicioi*, so the presence of the same or related linear diterpenoids seems probable.[4,5]

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Chemical studies on *Pterodon* have shown the presence of alkaloid compounds in the bark,[6] isoflavone and some triterpenes in the wood[7] and diterpenes[3,8] and isoflavones in seed oil.[9] Among other substances, 14 furan-diterpenes were described and isolated from the fruit of the *Pterodon* genus. In fact, four of these diterpenes belong to the *P. polygalaeflorus* Benth species.[9] The furan-diterpene 6α,7β-dihydroxyvouacapan-17 sodium-oate, isolated from the fruit oil of *P. polygalaeflorus* Benth, demonstrated an anti-inflammatory activity in paw oedema produced by carrageenan[10,11] and analgesic effects in the writhing test in mice.[12] Also, the hexanic crude extract obtained from *P. emarginatus* seeds inhibited the migration of neutrophils towards the peritoneal cavity of rats.[13]

P. emarginatus seeds are extensively used in Brazilian folk medicine and, to date, there is no scientific documentation concerning the antiulcerogenic and anti-inflammatory activities of their essential oil. In line with these findings, this work is especially relevant since it aims to evaluate the potential antiulcer and anti-inflammatory effects of the *P. emarginatus* essential oil. Therefore, this study investigates, by means of functional and biochemical approaches, the antiulcerogenic and anti-inflammatory activity using various in-vivo experimental peptic ulcer models and a model of pleurisy induced by carrageenan.

Materials and Methods

Drugs and reagents

The following drugs were used: carrageenan λ type IV (Sigma Chemical Co., St Louis, MO, US), omeprazole (AstraZeneca, Monts, France) and ranitidine (União Química Farmacêutica Nacional S.A. Laboratories, São Paulo, Brazil). Indometacin, celecoxib, ethanol, Tween 80 and dimethyl sulfoxide (DMSO) were all from Merck & Co. Inc. (White-house Station, US). Heparin was obtained from Roche (NJ, US; Liquemine), May-Grünwald dye from Newprov (Pinhais, PR, Brazil) and Giemsa dye from Laborclin (Pinhais, PR, Brazil). Other reagents used were of analytical grade and obtained from different commercial sources.

Plant material

The seeds of *Pterodon emarginatus* were collected in September 2006 in Três Marias (MG, Brazil) and authenticated by Dr Fátima Salimena from the Universidade Federal of Juiz de Fora (UFJF), Brazil. A voucher specimen (No. 48.077) has been deposited at the CESJ Herbarium/UFJF.

Isolation of the essential oil

The seeds of *Pterodon emarginatus* were triturated (30 g) and hydrodistilled in a Clevenger-type apparatus. After 2 h distillation, the essential oil was collected and the yield obtained was 3.9% (w/w).

Essential oil analysis

The oil constituents were analysed by GC/MS. The GC was performed in a HP6890N apparatus with DB5MS column (30 m × 0.25 mm i.d, with 0.25 μm film) using helium as carrier gas (1 ml/min). The column temperature was programmed for a 70–300°C range at 2°C/min, 280°C for 60 min. Temperatures of the injector and detector were 250°C and 280°C, respectively. Electron impact (EI) at ionization energy of 70 eV was recorded in a HP5973N with a mass selective detector. Sample components were identified by matching their mass spectra with those recorded in NIST/Wiley 275.1 library and comparing with previously reported data the of GC retention index.[14]

Animals

Male Swiss mice (*Mus musculus*), 25–30 g, obtained from the Reproduction Biology Center, UFJF, were housed in groups of five in standard cages at room temperature (25 ± 3°C) in a 12-h light–dark cycle, with both food and water freely available. The experimental mice were fasted for 12 h. The experimental protocol followed the principles and guidelines suggested by the Brazilian College of Animal Experimentation and were approved by the local ethical committee.

Antiulcerogenic activity

Ethanol-induced gastric ulceration

The experiment was performed according to Gupta et al.[15] with few modifications. The mice were randomly separated into five groups (n = 6/group). The first group was given saline (10 ml/kg), while the second group was treated with ranitidine (60 mg/kg). The remaining groups received 100, 300 and 500 mg/kg of essential oil of *P. emarginatus*, respectively. All the treatments were administered orally. One hour later, ulceration was induced by intragastric oral gavage of 0.2 ml of 99.5% ethanol. One hour after ethanol administration, the mice were sacrificed; the stomachs were surgically removed and opened along the greater curvature to examine the lesions macroscopically. The stomachs were rinsed with 0.9% saline and examined under a dissecting microscope to assess the formation of ulcers. The ulcers were graded using the following score system: 0, normal mucosa; 0.5, red coloration; 1, haemorrhagic streak or oedema; 2, ≤ 10 petechiae; 3, > 10 petechiae; 2 × (number of ulcers or erosions < 1 mm); 3 × (number of ulcers or erosions > 1 mm); 4 × (number of ulcers with perforation).[16] The mean ulcer score for each mouse was expressed as ulcer index (UI) and the inhibition percentage was calculated by the formula: $[(UI_{Control} - UI_{Treated})/UI_{Control}] \times 100$. Percentage ulcer inhibition was calculated for each group in comparison with the saline group.

Nonsteroidal anti-inflammatory drug (NSAID)-induced ulcer

As previously described by Raji *et al.*,[17] mice were randomly separated into five groups (n = 6/group). The first group received saline (10 ml/kg), the second group was treated with ranitidine (60 mg/kg) and the remaining groups received 100, 300 and 500 mg/kg of essential oil of *P. emarginatus*, respectively. All the treatments were administered orally. One hour after treatment, mice from all groups received indometacin (20 mg/kg) to induce gastric ulcer. Six hours later, the mice were sacrificed, the stomachs removed and opened along the greater curvature. The stomachs were rinsed with 0.9% saline and examined under a dissecting microscope

to assess the formation of ulcers. The images obtained were analysed using the parameters described previously.

HCl/Ethanol induced gastric ulceration

As previously described by Yesilada *et al.*,[18] the mice were randomly separated into five groups ($n = 6$/group). The first group received saline (10 ml/kg), the second group was treated with omeprazole (30 mg/kg) as standard control and the remaining groups received 100, 300 and 500 mg/kg of the essential oil of *P. emarginatus*, respectively. Thirty minutes after treatment, all the mice were orally treated with 0.2 ml of 0.3 M HCl/60%ethanol mixture to induce gastric ulcer. Mice were killed 1 h after administration of HCl/ethanol mixture and the stomachs were removed and opened along the greater curvature. The stomachs were rinsed with 0.9% saline and examined under a dissecting microscope to assess the formation of ulcers. The images obtained were analysed using the parameters described above.

Anti-inflammatory activity

Pleurisy induced by carrageenan

As previously described by Saleh *et al.*,[19] different groups of mice were orally treated with saline (10 ml/kg), essential oil of *P. emarginatus* (100, 300 and 500 mg/kg), indometacin (10 mg/kg) or celecoxib (18 mg/kg), 1 h before the induction of pleurisy. Pleurisy was induced by the single intrapleural injection of 0.1 ml of 1% carrageenan solution (w/v). Four hours after carrageenan injection, the mice were sacrificed and the pleural exudate was collected by pleural cavity lavage with 2.0 ml of sterile phosphate buffered saline containing heparin (20 IU/ml). Exudate volumes were measured and the results were calculated by subtracting the volume injected into the pleural cavity from the total volume recovered. Total leucocyte counts were performed in a Neubauer chamber. Cellular smears were prepared with 0.1 ml of pleural lavage to determine the differential leucocyte count. The slides were stained with May-Grünwald–Giemsa dye and the analysis was carried out using a light microscope.

NO level

Nitric oxide was measured indirectly by its products nitrite (NO_2^-) and nitrate (NO_3^-) using the Griess reaction.[20] Samples of the exudate were collected, homogenized in trichloroacetic acid–water (50% v/v) and centrifuged at 14 000 rev/min for 10 min. The supernatant was used for the quantification of NO by means of colorimetric measurements (540 nm) in a microplate reader (Organon Tecknica, Roseland, NJ, US). The results were expressed as μM.

Tumour necrosis factor (TNF)-α and interleukin-1 (IL-1)α levels

Samples of exudates were collected and immediately prepared for the analysis of cytokine levels. Commercially available kits were used with monoclonal specific antibodies for each cytokine (BD OptEIA, CA, US). All cytokine concentrations were estimated by means of colorimetric measurement (450 nm) in a microplate reader (SPECTRAMAX 190, Molecular Devices). The amount of cytokines was calculated from the standard curve, for the different concentrations of the recombinant cytokines.

Statistical analysis

The results are shown as the mean ± standard error of mean (SEM) of six mice per group. Analysis of variance followed by Bonferroni's test were performed and $P < 0.05$ was considered as being significant.

Results

Chemical composition of essential oil of *P. emarginatus*

The essential oil constituents were analysed by GC/MS. Eleven compounds were identified, most of which were *trans*-caryophyllene (35.9%), beta-elemene (15.3%), germacrene-D (9.8%), alpha-humulene (6.8%), spathulenol (5.9%) and bicyclo germacrene (5.5%). All compounds in the essential oil of *P. emarginatus* Vogel were identified as monoterpenes and sesquiterpenes (Table 1).

Antiulcerogenic activity

Ethanol-induced gastric ulceration

Number of ulcers and ulcer index were significantly attenuated by essential oil of *P. emarginatus* (at all tested doses) and ranitidine (60 mg/kg) compared with saline treatment ($P < 0.05$) (Table 2). Essential oil (at all tested doses) and ranitidine were potent inhibitors of ethanol-induced gastric ulceration (> 80 ± 4.64% inhibition and 66.42 ± 5.35%, respectively).

Nonsteroidal anti-inflammatory drug (NSAID)-induced ulcer

The treatment with essential oil of *P. emarginatus* (at all tested doses) and ranitidine (60 mg/kg) reduced significantly all the evaluated parameters in comparison with control group ($P < 0.05$) in the indometacin-induced ulcer model (Table 3). The inhibition of ulcers was 69.50 ± 0.74% at all tested doses of essential oil and 66.42 ± 5.35% for the ranitidine-treated group.

Table 1 Chemical composition of essential oil of *Pterodon emarginatus* seeds

Compound	Retention time (min)	KI	Composition (%)
δ-Elemene	15.64	1327	4.7
β-Elemene	15.67	1380	15.3
trans-Caryophyllene	18.16	1411	35.9
β-Gurjunene	18.49	1423	4.6
α-Humulene	19.23	1449	6.8
γ-Muurolene	19.44	1456	2.7
Germacrene-D	19.97	1475	9.8
Bicyclo germacrene	20.44	1492	5.5
Spathulenol	22.53	1570	5.9
Caryophyllene oxide	22.72	1577	3.8
cis-Farnesyl acetate	26.34	1815	4.9
Total			99.9%

KI, Kovats index.

Table 2 Effect of oral administration of *Pterodon emarginatus* essential oil and ranitidine on acute gastric ulcer induced by absolute ethanol in albino mice

Treatment	Dose (mg/kg)	No. of ulcers (mm^2)	Ulcer index (%)	% Ulcer inhibition
Saline	N.A.	63.00 ± 5.45	68.50 ± 4.95	N.A.
Essential oil	100	4.50 ± 3.36**	8.17 ± 3.21**	88.07
	300	5.33 ± 3.95**	8.50 ± 4.06**	87.60
	500	10.33 ± 5.71**	13.67 ± 5.95**	80.04
Ranitidine	60	19.17 ± 4.94**	23.00 ± 5.35**	66.42

Data are expressed as mean ± SEM, n = 6/group.
N.A., not applicable.
*$P < 0.05$, **$P < 0.001$ when compared with saline-treated mice (analysis of variance followed by Bonferroni's test).

Table 3 Effect of oral administration of *Pterodon emarginatus* essential oil and ranitidine on gastric ulcer induced by indometacin in albino mice

Treatment	Dose (mg/kg)	No. of ulcers (mm^2)	Ulcer index (%)	% Ulcer inhibition
Saline	N.A.	8.00 ± 1.71	12.33 ± 1.85	N.A.
Essential oil	100	1.00 ± 0.68**	3.67 ± 0.80**	70.23
	300	1.00 ± 0.44**	3.83 ± 0.60**	69.93
	500	1.67 ± 0.80**	4.67 ± 0.84**	62.12
Ranitidine	60	0.67 ± 0.42**	3.50 ± 0.50**	71.61

Data are expressed as mean ± SEM, n = 6/group.
N.A., not applicable.
*$P < 0.05$, **$P < 0.001$ when compared with saline-treated mice (analysis of variance followed by Bonferroni's test).

Gastric lesions induced by HCl/ethanol

A dose-dependent antiulcerogenic activity was observed in the treated groups after the oral administration of *P. emarginatus* seeds essential oil (Table 4). The inhibition of ulcers was 46.07 ± 2.39%, 78.82 ± 0.84%, 79.14 ± 0.71% and 82.80 ± 1.05% for the groups treated with 100, 300 and 500 mg/kg of essential oil of *P. emarginatus* and omeprazole, respectively.

Anti-inflammatory activity

Effect on carrageenan-induced pleurisy in mice

Essential oil of *P. emarginatus* (300 and 500 mg/kg) caused a marked reduction in the exudate volume (59 ± 0.03% and 61 ± 0.05%; Figure 1a), in the leucocytes migration (17 ± 1.13% and 46 ± 0.40%; Figure 1b) and in the number of polymorphonuclear leucocytes that migrated into the pleural cavity (63 ± 0.50% and 66 ± 0.19%; Figure 1c), respectively. On the other hand, it did not have an effect on the number of mononuclear leucocytes (Figure 1d). When comparing indometacin and celecoxib effects, a similar effect can be observed in the exudate volume and total leucocytes, and the essential oil showed a better activity in reducing the number of polymorphonuclear leucocytes (Figure 1).

Effect on blood cells

Essential oil (100, 300, 500 mg/kg), indometacin or celecoxib did not change the number of blood white or red cells in this model of pleurisy (data not shown).

NO levels

The pre-treatment (1 h) of mice with the essential oil caused a marked reduction in the NO levels (inhibition: 88 ± 0.09, 78 ± 0.03 and 82 ± 0.03%) (Figure 2a). The indometacin and celecoxib pre-treatments presented inhibitory effects on NO levels of 56 ± 8% and 39 ± 7% (Figure 2a).

TNF-α and IL-1α levels

Animals pre-treated (1 h) with the essential oil 100, 300 and 500 mg/kg presented a marked reduction in IL-1α levels (inhibition: 50.30 ± 8.39, 33.50 ± 6.98 and 37.57 ± 10.60%,

Table 4 Effect of oral administration of essential oil of *Pterodon emarginatus* and omeprazole on gastric ulcer induced by HCl/ethanol in albino mice

Treatment	Dose (mg/kg)	No. of ulcers (mm^2)	Ulcer index (%)	% Ulcer inhibition
Saline	N.A.	21.33 ± 2.74	25.17 ± 2.68	N.A.
Essential oil	100	12.33 ± 2.39*	14.33 ± 2.39*	46.07
	300	3.00 ± 0.03**	5.33 ± 0.84**	78.82
	500	2.83 ± 0.75**	5.25 ± 0.71**	79.14
Omeprazole	30	3.33 ± 1.12**	4.33 ± 1.05**	82.80

Data are expressed as mean ± SEM, n = 6/group.
N.A., not applicable.
*$P < 0.05$, **$P < 0.001$ when compared with saline-treated mice (analysis of variance followed by Bonferroni's test).

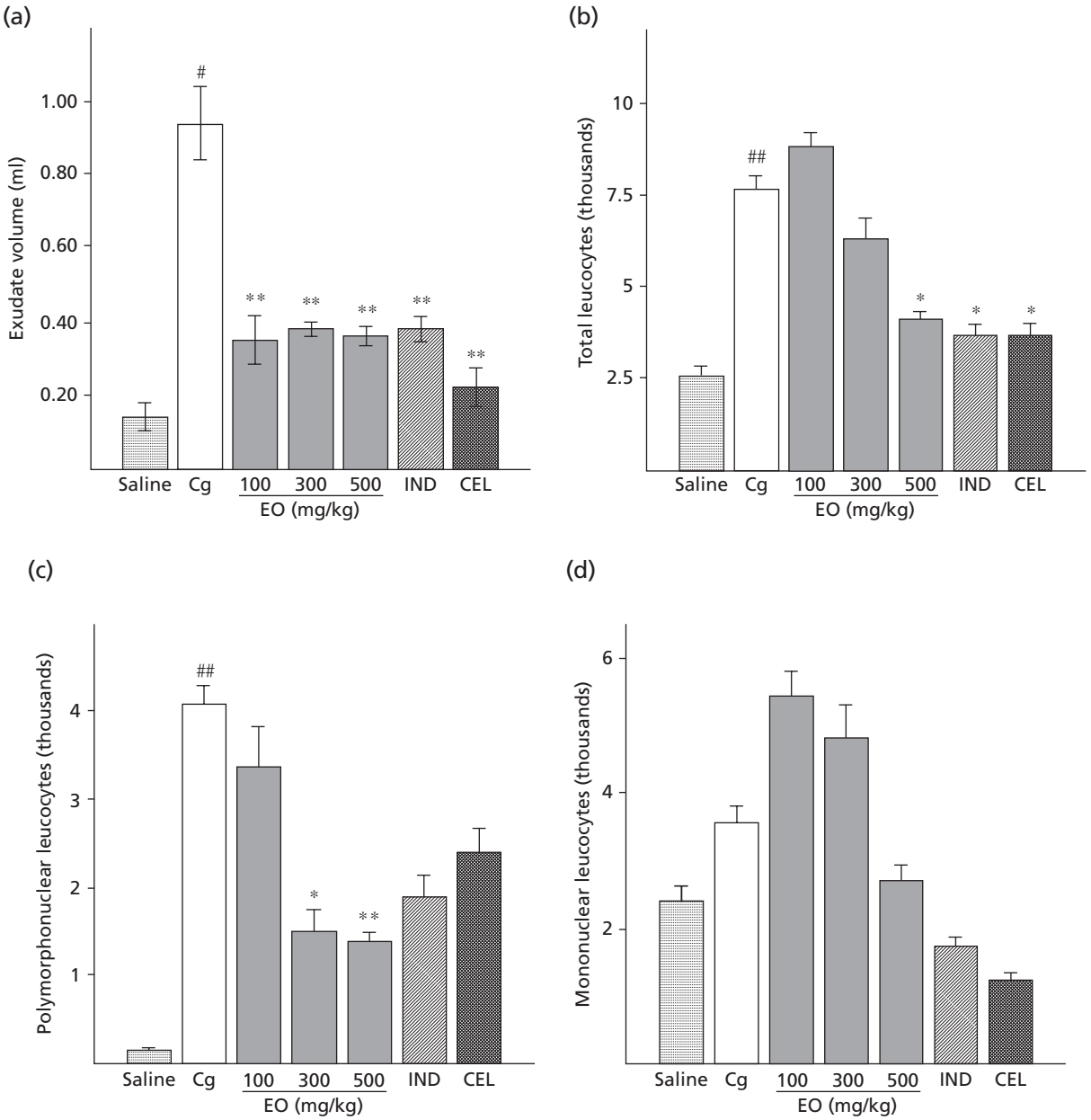

Figure 1 Effect of pre-treatment with essential oil (EO) of *Pterodon emarginatus* (100–500 mg/kg, p.o., 1 h before carrageenan) on exudation (a), number of total leucocytes (b) and polymorphonuclear (c) and mononuclear (d) leucocytes on the mouse model of pleurisy induced by carrageenan. Saline, response in mice treated only with sterile saline (0.9% NaCl); Cg, response in mice treated only with carrageenan (1 mg/cavity, 4 h); IND, response in mice treated with indometacin (10 mg/kg, p.o., 1 h) plus carrageenan; CEL, response in mice treated with celecoxib (18 mg/kg, p.o., 1 h) plus carrageenan. Each group represents the mean of four to six mice and the vertical lines show the SEM. $^{*}P < 0.05$, $^{**}P < 0.001$ when compared with carrageenan-treated mice; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ when compared with saline-treated mice (analysis of variance followed by Bonferroni's test).

respectively; $P < 0.001$) compared with control group (Figure 2c). The celecoxib pre-treatment prevented an increase in the levels of IL-1 by 46.00 ± 8.31% ($P < 0.001$) (Figure 2c). On the other hand, it had no effect on the TNF-α level (Figure 2b).

Discussion

Proton pump inhibitors and H_2 receptor antagonists are available to treat gastrointestinal diseases, but clinical evaluation of these drugs showed an incidence of relapses, side effects and drugs interactions.[21,22] Thus, the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs which offer better protection and decreased relapse.

Gastrointestinal injury can be induced in experimental animals by various chemical agents,[23] and ethanol-induced gastric ulcers in mice and rats have been widely used for the evaluation of gastroprotective activity. Ethanol induces ulcers by the reduction of gastric mucosal blood flow and

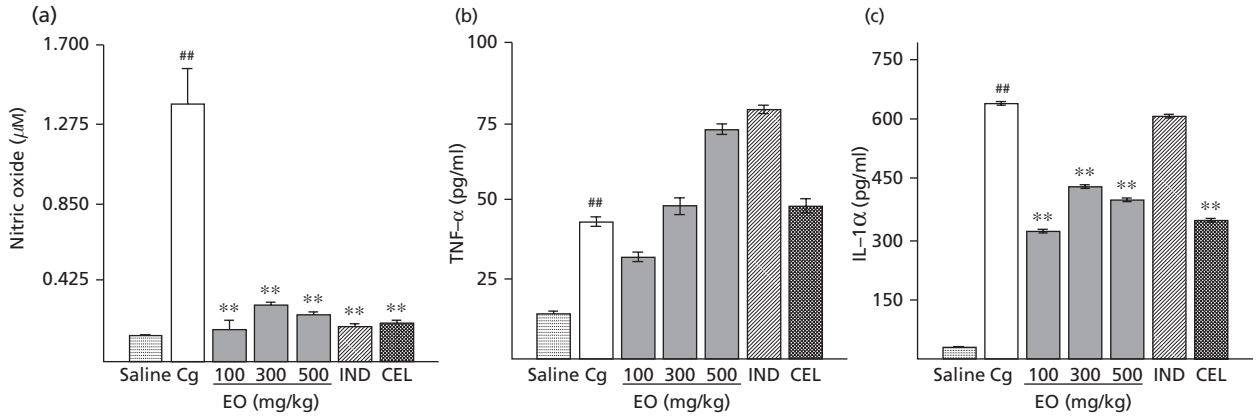

Figure 2 Effect of pre-treatment with essential oil (EO) of *Pterodon emarginatus* (100–500 mg/kg, p.o., 1 h before carrageenan) upon nitric oxide (a), TNF-α (b) and IL-1α (c) levels in the mouse model of pleurisy induced by carrageenan. Saline, response in mice treated only with sterile saline (0.9% NaCl); Cg, response in mice treated only with carrageenan (1 mg/cavity, 4 h); IND, response in mice treated with indometacin (10 mg/kg, p.o., 1 h) plus carrageenan; CEL, response in mice treated with celecoxib (18 mg/kg, p.o., 1 h) plus carrageenan. Each group represents the mean of four to six mice and the vertical lines show the SEM. $^{*}P < 0.05$, $^{**}P < 0.001$ when compared with carrageenan-treated mice; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ when compared with saline-treated mice (analysis of variance followed by Bonferroni's test).

mucus production in the gastric lumen, a decrease in endogenous glutathione as prostaglandin (PG) levels and an increase of ischaemia, gastric vascular permeability, acid 'back diffusion', histamine release, efflux of sodium and potassium, influx of calcium, generation of free radicals and production of leukotrienes (LT).[24] These data suggest that antioxidant compounds could be active in this experimental model, producing antiulcerogenic effects or cytoprotection.

In this study, the control group treated orally with ethanol clearly presented the expected characteristic zone of necrotizing mucosal lesions. On the other hand, treatments with the essential oil of *P. emarginatus* significantly reduced the ulcer index and the number of lesions.

Nonsteroidal anti-inflammatory drugs (NSAIDs), like aspirin and indometacin, are known to induce ulcers during the course of anti-inflammatory therapy, by inhibiting PG synthesis through the cyclooxygenase (COX) pathway.[25] In the stomach, PGs play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair.[26] Thus, the suppression of PG synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastroduodenal ulceration. It was observed that the essential oil of *P. emarginatus* significantly prevented the mucosal damage in the indometacin-induced ulcer model. These results suggest the possible involvement of PGs or mucus in the antiulcerogenic effect produced by this oil.

Chloridric acid–ethanol mixture induced gastric damage in mice possibly through LT production and also involvement of 5-lipooxygenase (5-LOX) in the formation of ulcer lesions. So the protective effect of the essential oil against the gastric damage might be due to protection against 5-LOX or LT pathways. It is well known that HCl–ethanol-induced gastric lesions are not inhibited by antisecretory agents like ranitidine, but are inhibited by agents that enhance mucosal defence factors such as omeprazole.[27]

A predominance of sesquiterpenes was found in the chemical composition of the essential oil from *P. emarginatus* seeds: α-humulene, β-elemene, germacrene-D, spathulenol, bicyclo germacrene, *cis*-farnesyl acetate and *trans*-caryophyllene (being in highest concentration). These findings confirm the data of Carvalho,[28] even though the presence of α-caryophyllene, diterpenes[3,8] and isoflavones was not verified in the essential oil of the seeds of *P. emarginatus*.[9] According to Abreu Gonzaga *et al.*,[29] bicyclogermacrene has anti-inflammatory and antibacterial activity and Fernandes *et al.*[30] demonstrated that oral administration of *trans*-caryophyllene and α-humulene significantly inhibited the expression of inducible nitric oxide synthase (iNOS) by carrageenan injection in the rat paw.

Inappropriate iNOS expression is known to be involved in the development of inflammatory alterations, including gastrointestinal tract and central nervous system diseases, ischaemia and lung inflammation and fibrosis, among others.[31–33] Therefore, drugs that modulate the expression or activation of iNOS could represent a promising and practical approach for the management of inflammatory diseases. The results showed a significant antiulcerogenic activity of the essential oil of *P. emarginatus* seeds via enhancement of antioxidant potential. Sesquiterpenes, as bicyclogermacrene, *trans*-caryophyllene and α-humulene, present in the essential oil may be responsible for this activity.

Inflammation is a complex process that is frequently associated with pain and involves several events, such as the increase of vascular permeability, increase of granulocytes and mononuclear cell migration, as well as granulomatous tissue proliferation. Carrageenan-induced pleurisy has been extensively used to investigate the mechanisms involved in acute inflammation and also to assess the effectiveness of anti-inflammatory drugs.[34] As expected, in our experiments, the intrapleural injection of carrageenan caused a marked accumulation of pleural exudate followed by an intense migration of inflammatory cells to the pleural cavity in the saline group. Mice treated with the essential oil presented a reduction in the volume of pleural exudate accumulated in response to carrageenan injection. Moreover, the oral

administration of the essential oil also inhibited the migration of total and polymorphonuclear leucocytes, although it had no effect in decreasing the number of mononuclear cells in the pleural cavity.

Generally, neutrophils are the first line of defence of the immunological system against pathogens. However, in several inflammatory diseases, such as rheumatoid arthritis, they represent a potential cause of tissue damage. Indeed, the interaction of recruited neutrophils at the site of inflammation with resident cells, local inflammatory mediators or extracellular matrix may lead to the production of several other mediators, including cytokines/chemokines, degrading enzymes, oxygen and nitrogen species and metalloproteases, that may further amplify the inflammatory response and injure the surrounding tissue.[35] In this study, we demonstrated for the first time that essential oil of *P. emarginatus* seeds has a potent capacity to reduce the number of leucocytes at the site of inflammation and showed an anti-inflammatory effect.

In the model of pleurisy induced by carrageenan the enhancement of nitrate levels represents the sum of NO production following the stimulation of both constitutive and inducible iNOS by different cells of the pleural cavity.[36] Furthermore, NO also modulates neutrophils *in vitro* by influencing the expression of the adhesion molecules endothelial selectin type E (E-selectin) and intercellular adhesion molecule type 1 (ICAM-1).[37] Since NO activity is an indirect marker of activated leucocytes and is implicated in exudation and cell migration,[38,39] the low NO levels following essential oil treatment could also be explained by the inhibition of leucocyte influx.

There is evidence that pro-inflammatory cytokines such as TNF-α and IL-1 help to propagate the extension of a local or systemic inflammatory process.[40] In animal models TNF-α and IL-1α are pivotal players in arthritis in several animal species, including rats, mice and rabbits.[41,42] Our results demonstrated that the essential oil (100 mg/kg) was more effective than celecoxib in inhibiting the levels of pro-inflammatory IL-1α. However, the essential oil failed to inhibit the TNF-α level.

In the studies concerning the mechanism of inflammation, the L-arginine–NO pathway has been proposed to play an important role in the carrageenan-induced inflammatory response.[43,44] Our results confirm that the carrageenan-induced paw oedema model results in the production of NO. In our study, the level of NO was decreased significantly by the treatment with essential oil of *P. emarginatus*. We suggest the anti-inflammatory mechanism of the essential oil may be through the L-arginine–NO pathway since it significantly inhibited the NO production and decreased the number of leucocytes migrating to the site of inflammation. The precise mechanism involved in its action is currently not completely understood but from this finding it is believed that the anti-ulcer and anti-inflammatory effects of the essential oil arises from its capacity to inhibit the release of inflammatory mediators and decrease the migration of leucocytes to the site of inflammation.

Therefore, the inhibitory effect of the essential oil upon leucocyte influx in the model of inflammation of the pleural cavity in mice induced by carrageenan demonstrated herein can be attributed to the suppression of NO release and pro-inflammatory cytokines such as IL-1, which is responsible for stimulating cellular chemotaxis and inducing the expression of adhesion molecules.[45] This activity can be explained, at least in part, by the presence of the sesquiterpenes α-humulene and *trans*-caryophyllene in this essential oil but the possibility of synergy between other constituents present in the oil cannot be excluded. These constituents were evaluated by several authors and, interestingly, an anti-inflammatory activity for such constituents was shown.[30,46,47]

Conclusions

The data obtained in this study revealed pronounced oral antiulcerogenic and anti-inflammatory properties of the essential oil of *Pterodon emarginatus* according to the assessment of several models of gastric ulcers. Therefore, it seems reasonable to suggest that essential oil of *P. emarginatus* or its constituents may represent, in the near future, a new therapeutic option for the treatment of inflammatory diseases, especially those presenting a chronic profile such as gastric ulcers. In addition, the results may justify the use of *P. emarginatus* in traditional medicine. Further studies aiming to investigate which compounds in the essential oil are responsible for the described antiulcerogenic and anti-inflammatory activity and the precise mechanisms of action are underway.

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Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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