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Acute and topic anti-edematogenic fractions isolated from the seeds of *Pterodon pubescens*

M. C. C. Silva, C. R. M. Gayer, C. S. Lopes, N. O. Calixto, P. A. Reis, C. P. B. Passaes, M. C. Paes, S. R. Dalmau, K. C. C. Sabino, A. R. Todeschini and M. G. P. Coelho

Abstract

We previously demonstrated that alcoholic extracts from *Pterodon pubescens* Benth. (Sucupira branca, Leguminosae) seeds exhibit anti-arthritic activity. In the present work we show that the oleaginous extract obtained from *P. pubescens* seeds (OEP) exhibits acute or topic anti-edematogenic activity when tested in carrageenan-induced paw edema or in croton oil-induced ear edema assays, respectively. Four fractions were obtained from OEP by sequential liquid–liquid extraction. The anti-edematogenic properties were predominant in the hexanic fraction, which was further fractionated by HPLC, yielding three sub-fractions (PF1.1, PF1.2 and PF1.3). PF1.1 and PF1.3 showed potent acute and topic anti-edematogenic activity. The PF1.2 sub-fraction, although not active in the carrageenan assay, exhibited a potent anti-edematogenic activity in the croton oil-induced ear edema. This sub-fraction shows a maximum efficacy similar to indometacin in a lower dose. The PF1.1 sub-fraction presented a complex mixture containing furane diterpene derivatives of vouacapan. PF1.2 consists of a single substance, geranylgeraniol, as determined by GC/MS and NMR, while PF1.3 contains farnesol.

Departamento de Bioquímica, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Av Professor Manoel de Abreu, 44, PAPC, 4º andar, CEP 20550-170, Rio de Janeiro, RJ, Brazil

M. C. C. Silva, C. R. M. Gayer, C. S. Lopes, N. O. Calixto, P. A. Reis, C. P. B. Passaes, M. C. Paes, S. R. Dalmau, K. C. C. Sabino, A. R. Todeschini, M. G. P. Coelho

Division of Experimental Medicine, Research Division, National Cancer Institute, Praça Cruz Vermelha 23, 6º andar, CEP 20230-130, Rio de Janerio, RJ, Brazil

S. R. Dalmau

Correspondence: M. G. P. Coelho, Universidade do Estado do Rio de Janeiro – Centro Biomédico, Instituto de Biologia Roberto Alcântara Gomes – Departamento de Bioquímica, Av Professor Manoel de Abreu, 44, PAPC, 4º andar, CEP 20550-170, Rio de Janeiro, RJ, Brazil.
E-mail: marsen@uerj.br

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Introduction

Chronic inflammatory diseases, such as rheumatoid arthritis, are still one of the main health problems of the world's population. Although several drugs are currently available to treat rheumatoid arthritis, prolonged use should be avoided because of their severe side effects (Lee & Weinblatt 2001). Alcoholic extracts from *Pterodon pubescens* Benth. (Sucupira branca) seeds are widely used in folk medicine throughout central Brazil for their anti-rheumatic, analgesic and anti-inflammatory properties (Pio Correa 1984). The anti-arthritic activity of the hydroalcoholic extract of *P. pubescens* seeds (HEPp) in collagen II-induced arthritis (CIA) was experimentally demonstrated by Sabino et al (1999a). Toxicological studies demonstrated that *P. pubescens* seed oil (OEP) did not present an acute toxicity effect in healthy mice after oral administration of high doses (Sabino et al 1999b). Furthermore, prolonged administration of anti-arthritic doses of HEPp did not induce any detectable subacute toxic side effect in mice with CIA (Coelho et al 2001).

It has been suggested that the anti-arthritic activity of *P. pubescens* is due to its anti-inflammatory and/or immunosuppressive activities (Sabino et al 1996, 1999a). The anti-inflammatory activity of OEP could be attributed, at least in part, to the presence of furan-diterpenes possessing a vouacapan skeleton (isolated by Fascio et al 1976), since these compounds, isolated from *P. polygalaeflorus* Benth. seed oil (another species of sucupira), reduced rat paw edema induced by carrageenan, serotonin and histamine (Nunan et al 1982). Despite the presence of vouacapan derivatives in OEP, its acute anti-inflammatory properties have not yet been demonstrated.

In the present work, we evaluate the acute and topic anti-edematogenic activity of OEP and its fractions using the carrageenan-induced paw edema and croton oil

(CO)-induced ear edema assays, respectively, and we demonstrate that compounds other than vouacapan derivatives contribute to the anti-edematogenic properties of OEP.

Materials and Methods

Chemicals

All reagents used were high-grade purity. Ethanol, methanol, hexane, dichloromethane and ethyl acetate were purchased from Merck (Brazil; HPLC grade). Indometacin, carrageenan λ grade IV, Tween 20 and croton oil were purchased from Sigma Chemical Company (USA). Deuterochloroform (CCl_3D) was acquired from Tedia Brazil Ltd and phenobarbital (Gardenal) from Aventis Pharma Ltd (Brazil).

Animals

Male SW mice 12–15 weeks old, 30–40 g body weight (b.w.), maintained with water and fed ad libitum were used in all experiments. For each experiment, mice were randomly selected into groups comprising 7–10 per cage. All experiments were performed under the consent and surveillance of the Biomedical Centre Ethical Committee of UERJ for the use of animals in research.

Plant extract and fractions

P. pubescens seeds were obtained from the State of Goiás, Brazil. The taxonomic identity of the respective tree was confirmed by Dr Haroldo Cavalcante de Lima from the Department of Systematic Botanics, Jardim Botânico do Estado do Rio de Janeiro, Brazil, where a voucher specimen has been deposited (RB 350279, 06-1999). Powdered seeds (50.02 g) were extracted at room temperature with absolute ethanol for 15 days under dark conditions. Solvent was removed under vacuum to obtain OEP, which was sequentially fractionated by liquid–liquid extraction with hexane, dichloromethane and ethyl acetate, yielding fractions PF1 (29.89 g), PF2 (23.89 g), PF3 (0.23 g) and PF4 (residue, 0.028 g), respectively. PF1 was submitted to HPLC in a Shimadzu system (Lichrosorb 10 RP-8 column, Phenomenex, USA; 1 mL min^{-1}, 70–100% methanol:H_2O in 17 min, followed by 100% methanol isocratic for 13 min) yielding PF1.1 (76%), PF1.2 (9%) and PF1.3 (15%) sub-fractions. These sub-fractions were monitored by absorbance at 254 nm and collected manually.

GC/MS

OEP crude extract, and PF1 fraction and sub-fractions were analysed by gas–liquid chromatography–mass spectrometry (GC/MS). The GC was performed in a Shimadzu 17A apparatus with a DB-1 fused-silica column (30 m × 0.25 mm i.d.) using hydrogen as the carrier gas. The column temperature was programmed for 120–300 °C at 5 °C min^{-1}, then 300 °C for 10 min. Electron impact at an ionization energy of 70 eV and chemical ionization by ammonium mass spectra were recorded in a Shimadzu QP 5050A with a mass selective detector. The mass spectral fragmentations obtained were compared to the data banks of Nist and Wiley.

NMR

NMR spectra (Cavanagah et al 1996) were obtained using a Brucker DRX 600 apparatus with a 5 mm triple resonance probe. PF1.2 was dissolved in 0.5 mL of CCl_3D before analysis. Proton NMR spectra were assigned through a COSY spectrum. Carbon chemical shifts were assigned through heteronuclear correlation (HMQC) recorded with full proton decoupling.

Carrageenan-induced mouse paw edema

The carrageenan assay procedure was carried out according to the method described by Levy (1969) with modifications. Mice fasted for 1 h with free access to water were randomly selected to perform one of the study groups: control, indometacin (10 mg kg^{-1} b.w.), OEP (40 μg, 200 μg and 20 mg kg^{-1} b.w.), PF1, PF2, PF3 and PF4 (2, 20 and 200 μg kg^{-1} b.w.), PF1.1, PF1.2 and PF1.3 (0.1, 1.0 and 10 μg kg^{-1} b.w.). Extract and solutions of fractions/sub-fractions were prepared in 15% ethanol containing 1.25% Tween 20 (vehicle). One hour after the i.p. administration of test solutions or vehicle (control), edema was induced by a subplantar injection of 50 μL of 0.6 g% (w/v) carrageenan suspension in physiological saline into the right hind paw of each mouse. The swelling of the carrageenan-injected foot was measured 3 h later in a pletismometer (7150 Ugo Basile) and compared with the volume of the same foot at the time of the carrageenan challenge (edema index).

CO-induced edema

Topical anti-inflammatory activity was evaluated by the inhibition of the CO-induced ear edema in mice (Tubaro et al 1985). Mice received a sedative i.p. dose of 4 mg kg^{-1} b.w. of phenobarbital. Cutaneous inflammation was induced by application of 10 μL of a 0.5% solution of the irritant (CO) in acetone. Variable amounts of the samples under test, or indometacin, were applied on the internal surface of the right ear, the left one receiving the vehicle. Control animals received only the phlogistic agent. The mice were sacrificed 6 h later (the peak of inflammation), and a plug of 7 mm in diameter was removed from each ear. The edema index was evaluated by measuring the differences in weight between the two plugs. The percentage inhibition of edema formation was determined by comparing the edema index of the treated group with that of the control animals.

Statistical analysis

The statistical significance of the data was determined by Tukey unequal N post-hoc tests. Differences between groups were considered significant at a level of $P \leq 0.05$.

Results

As shown in Table 1, carrageenan injection in control mice resulted in a prominent paw edema of 102.70 ± 32.37% after 3 h (peak of inflammation). The i.p. injection of animals with OEP caused a dose-dependent inhibition of carrageenan-induced inflammation (EC50 = 15.12 $\mu g\,kg^{-1}$, $R^2 = 0.90$), showing the edema inhibition index of 43% as the best (20 $mg\,kg^{-1}$). Indometacin (10 $mg\,kg^{-1}$), used as the standard drug, imputed an inhibition of 62.14%.

Searching for compounds exhibiting anti-inflammatory activity, we performed a sequential liquid–liquid extraction of OEP with hexane, dichloromethane and ethyl acetate, obtaining PF1 (55%), PF2 (43%), PF3 (1.4%) and the residue PF4 (0.6%), respectively. When these fractions were tested on the carrageenan-induced mouse paw edema, only PF1 exhibited a dose-dependent acute anti-edematogenic response with the best inhibition of edema index at 200 $\mu g\,kg^{-1}$ b.w. (56.5%, Table 1), with an EC50 of 0.34 $\mu g\,kg^{-1}$ ($R^2 = 0.95$).

Further purification of PF1 by HPLC yielded PF1.1 (76%), PF1.2 (9%) and PF1.3 (15%) sub-fractions. When assayed in the carrageenan-induced paw edema model, PF1.1 showed a significant inhibitory activity only at the 0.1 $\mu g\,kg^{-1}$ b.w. dose (48.3%, Table 1). Still in this model PF1.3 exhibited this activity (EC50 = 0.88 $ng\,kg^{-1}$, $R^2 = 0.98$) in the range of 0.01 to 10 $\mu g\,kg^{-1}$ b.w. doses ($P < 0.05$), showing inhibition indexes in the range of 33.6–37.7%. PF1.2 lacked anti-inflammatory activity.

The anti-edematogenic effects of these extracts were also investigated by the CO-induced ear edema model to evaluate their topical activity. The treatment of animals with OEP doses ranging from 0.00042 to 420 $\mu g\,ear^{-1}$ resulted in significant inhibition indexes (EC50 = 0.15 $\mu g\,ear^{-1}$, $R^2 = 0.76$) of the edema ranging from 38.8 to 59.5% (Table 2). In a similar way, PF1 caused a significant

Table 1 Acute anti-inflammatory effect of OEP, its fractions and indometacin on carrageenan-induced paw edema.

Groups	Dose ($\mu g\,kg^{-1}$)[a]	n	% edema ± s.d.[b,d]	% inhibition[c]
Control	–	42	102.70 ± 32.37	–
OEP	0.4	10	98.85 ± 27.91	9.61
	40	10	68.86 ± 20.91*	32.95
	200	10	70.55 ± 16.24*	31.30
	20 000	9	58.11 ± 20.18**	43.41
PF1	0.002	10	86.65 ± 20.78	15.65
	0.02	10	85.76 ± 12.38	16.51
	0.2	10	74.56 ± 19.13	27.41
	2.0	10	51.88 ± 12.17**	49.48
	20	11	56.90 ± 16.82**	44.59
	200	10	44.67 ± 16.16**	56.50
PF1.1	0.001	10	77.97 ± 20.40	24.10
	0.1	10	53.10 ± 21.68**	48.29
	1.0	10	78.57 ± 23.41	23.49
	10	10	82.01 ± 12.01	20.14
PF1.2	0.1	10	86.14 ± 21.41	16.12
	1.0	10	134.00 ± 42.59	−30.47
	10	10	96.00 ± 27.39	6.52
PF1.3	0.001	10	88.64 ± 18.39	13.70
	0.01	10	69.12 ± 17.72	32.71
	0.1	10	68.18 ± 22.42*	33.61
	1.0	10	64.02 ± 22.07*	37.66
	10	10	64.68 ± 13.91*	37.02
PF2	2.0	7	115.40 ± 11.38	−12.36
	20	7	98.56 ± 17.41	4.03
	200	7	115.80 ± 28.60	−19.97
PF3	2.0	7	111.60 ± 32.18	−8.66
	20	7	78.30 ± 31.15	23.75
	200	7	81.16 ± 28.28	20.97
PF4	2.0	7	84.98 ± 30.86	17.25
	20	7	100.30 ± 17.46	2.33
	200	7	105.90 ± 29.96	−3.11
Indometacin	10 000	10	38.88 ± 10.00**	62.14

n = number of animals per group. [a]Doses were administered i.p. Results were expressed as [b]the increase in paw volume in relation to t_o (± s.d.) and [c]the percentage of anti-inflammatory activity. [d]Paw volume was measured 3 h after carrageenan injection (50 μL of 0.6 g%). *$P < 0.05$, **$P < 0.01$ compared with control group (Tukey unequal N post-hoc tests).

Table 2 Topical anti-inflammatory effect of OEP, its fractions and indometacin on CO-induced ear edema.

Groups	Dose (μg 20 μL^{-1})	n	% edema ± s.d.[a,c]	% inhibition[b]
Control	–	14–22 per group	68.57 ± 2.70[d]	–
Indometacin	450	11–15 per group	19.11 ± 1.40[d]***	72.13
OEP	0.00042	14	40.78 ± 9.92*	38.87
	0.042	13	37.18 ± 11.24*	44.28
	4.2	12	29.57 ± 22.58**	55.68
	42	12	34.16 ± 23.72**	48.80
	420	12	27.01 ± 18.21***	59.52
PF1	0.0056	14	44.26 ± 11.37*	39.26
	0.056	13	37.42 ± 13.16**	48.66
	0.56	15	27.89 ± 20.22***	61.73
	5.6	15	31.27 ± 29.71***	57.10
	56	15	38.63 ± 25.31**	47.00
PF1.1	0.004	15	48.38 ± 9.50	25.57
	0.04	15	39.33 ± 16.30**	36.01
	0.4	15	42.37 ± 14.14**	34.92
	4.0	15	44.78 ± 14.14**	31.11
PF1.2	0.00008	14	48.66 ± 19.75	28.63
	0.0008	14	38.59 ± 13.39**	43.40
	0.008	14	28.20 ± 9.03**	58.73
	0.08	14	24.96 ± 15.03**	62.69
	0.8	14	38.94 ± 23.74**	43.04
PF1.3	0.00006	14	42.90 ± 7.10**	38.74
	0.0006	14	33.39 ± 16.96***	52.31
	0.006	15	30.98 ± 11.26***	55.74
	0.06	15	39.94 ± 15.45***	42.94
	0.6	15	45.20 ± 13.44***	35.43
Vehicle (EtOH)	–	6	70.17 ± 21.72	1.92

n = number of animals per group. Results were expressed as [a]increase in ear plug weight (± s.d.) and [b]the percentage of topical anti-inflammatory activity, calculated using the control group of each experiment. [c]Edema index measured 6 h after CO application (20 μL of 0.5%) was calculated using the control group of each experiment. [d]Data represents the mean ± s.d. of the control and indometacin used in each treatment group. *$P < 0.01$, **$P < 0.001$, ***$P < 0.0001$ compared with control group by the Tukey unequal N post-hoc tests.

inhibition (EC50 = 0.03 μg ear^{-1}, $R^2 = 0.59$) of CO-induced ear edema when tested in doses ranging from 0.0056 to 56 μg ear^{-1}, showing an edema inhibition level similar to OEP. All PF1.1, PF1.2 and PF1.3 sub-fractions induced significant reduction of the topical edematogenic response (Table 2). Although the higher inhibition index has been observed with PF1.2 (62.7%), with a sigmoid dose-dependent response (EC50 = 0.046 ng ear^{-1}, $R^2 = 0.71$), significant inhibition was observed for PF1.1 and PF1.3 in almost all doses tested, with an inverted U-shaped dose response curve. The higher inhibition indexes of the CO-induced ear edema obtained with the topical application of OEP and its fractions were all comparable ($P > 0.05$) to that of the reference drug (Table 2).

The chromatographic profiles from PF1.1, PF1.2 and PF1.3 sub-fractions were obtained by GC/MS. The major components of these sub-fractions, determined with the mass-spectral fragmentation data banks of Wiley and Nist, are listed in Table 3. In PF1.1 two isomers of methyl 6,7-dihydroxyvouacapa n-17-oate were identified. PF1.2 exhibited only one peak. The 1H NMR analysis of PF1.2 showed a singlet at 1.60 ppm (9H), a singlet at 1.68 ppm (6H), two singlets at 2.00 and 2.05 ppm (10 H), a doublet at 4.15 (J = 7 Hz; 2H), two protons at 5.13 ppm and a triplet at 5.52 ppm (1H), which is in agreement with the presence of geranylgeraniol as previously described (Mors et al 1967; Santos et al 1972). PF1.3 is a complex mixture from which we were able to identify only farnesol, although we could not determine which of the isomers was present.

Discussion

In this work we used the carrageenan-induced paw edema and the CO-induced ear edema in the mouse, common models in the study of anti-inflammatory activities, to evaluate the putative anti-edematogenic properties of OEP and its fractions (Sugishita et al 1981; Tubaro et al 1985; Morris 2003).

Carrageenan injection into mouse paw evokes a potent local acute inflammatory reaction (Levy 1969) with a bi-phasic profile (Henriques et al 1987). We evaluated the anti-edematogenic properties of the OEP and its fractions over 3 h, during the first phase of this response in mice, which reaches a maximal edema development at 2–4 h. Afterwards it shows a slight decrease up to 24 h, when the second phase

Table 3 Components of PF1.1, PF1.2 and PF1.3 as determined by GC/MS.

Fraction	Retention time	M^+	Mass spectral data (m/z)	Substance
PF1.1	11.16	204	204(3), 189(2), 177(5), 161(5), 153(10), 147(5), 133(20), 119(60), 105(90), 91(75), 79(30)	naphthalene
	15.00	222	204(3), 189(2), 177(5), 161(5), 153(10), 147(5), 135(25), 119(25), 107(30), 93(70), 81(20), 69(100)	dimethyl-dodecatrienol
	35.12	390	372(3), 344(30), 330(5), 326(10), 312(80), 285(10), 267(10), 178(80), 145(35), 131(90), 123(30), 119(30), 109(27), 43(100)	methyl-dihydroxy-vouacapanoate
	35.60	390	372(5), 344(20), 330(1), 326(10), 312(90), 285(30), 267(30), 178(20), 145(35), 131(90), 123(30), 119(30), 109(27), 43(100)	methyl-dihydroxy-vouacapanoate
PF1.2	25.37	290	221(1), 202(1), 189(1), 161(30), 137(20), 135(35), 121(30), 107(20), 93(80), 69(100)	geranylgeraniol
PF1.3	18.75	260	249(1), 221(2), 204(5), 189(15), 175(3), 161(20), 149(15), 136(80), 121(60), 107(70), 93(90), 69(95), 67(85), 43(100)	farnesol

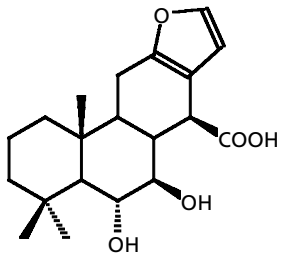
begins. Neutrophils are the predominant homing cells in the first phase, while macrophages, eosinophils and lymphocytes predominate in the second phase (Henriques et al 1987).

The 2–5 h interval of the first phase in the carrageenan mouse model is very sensitive to cyclooxygenase inhibitors (COX) (Sugishita et al 1981; Herencia et al 1998; Nishikori et al 2002). The anti-edematogenic properties of OEP/fractions could therefore be related to the inhibition of the synthesis of arachidonic acid metabolites. In support of this hypothesis, in experiments performed in our laboratory OEP inhibited platelet aggregation induced by arachidonic acid in rabbit plasma (data not shown).

The advantages of the CO-induced ear inflammation model are its good predictive value for screening topical anti-inflammatory activity and its sensitivity to both steroidal and non-steroidal drugs (SAIDs and NSAIDs, respectively). However, this sensitivity is dependent on the time course of the response (Tubaro et al 1985). Our results, using low CO doses (0.6 g%), were all evaluated at the peak of the first response phase (6 h). In this phase the most important mediators involved are prostaglandins, whose synthesis is responsive to both SAIDs and NSAIDs, histamine and serotonin, whereas the lipoxygenase pathway plays no important role (Tubaro et al 1986; Chen et al 1994). Again, the anti-edematogenic properties of the OEP, PF1 and sub-fractions could be mediated by a reduction in COX product synthesis, as reported for other anti-edematogenic agents (Blazsó & Gábor 1995; Siqueira-Junior et al 2003).

As stated above, neutrophils are the predominant infiltrating cells in the phase of carrageenan-induced paw edema studied here. These cells are the source of a myriad of inflammatory mediators. OEP substances could therefore be impairing the homing of these or other cells to the injured site, showing an anti-inflammatory and not just anti-edematogenic effects, as evaluated by its effects on edema models. Such a hypothesis is supported by our observation in the CIA model, where HEPp treatment, besides impairing disease development, drastically reduces the articular leukocyte infiltration (unpublished observations). Additionally, preliminary experiments using the mouse carrageenan-induced pleurisy model revealed a significant ($P < 0.05$) inhibition of the total cell counts in the pleural cavity following treatments with PF1, PF1.1 and PF1.2 at the inhibitory doses ($2\,\mu g\,kg^{-1}$, $0.1\,\mu g\,kg^{-1}$ and $0.1\,\mu g\,kg^{-1}$, respectively) used in the carrageenan-induced paw edema model (unpublished results).

Our results reveal that a high grade of purification has been achieved by the OEP fractionation procedures used here, as underlined by the low EC50 values of isolated fractions, compared to that of the OEP. The achievement of PF1.1 in obtaining anti-edematogenic effects in low rather than high doses of extracts is not unusual in phytopharmacology (Maleki et al 2001). One hypothesis is that the natural anti-inflammatory substance(s) present in extracts might exhibit pro-inflammatory activity when used in high amounts or that fractions might carry components with either anti- or pro-inflammatory effects. The vouacapan derivative 6,7-dihydroxyvoua capan-17-oic acid (Fascio et al 1976; Figure 1), found in PF1.1, together with other fractions such as 6α-hydroxyvouacapan-7β-17β-lactone or 6α,7β-dihydroxyvouacapan-17β-oate, present



6α, 7β-dihydroxyvouacapan-17β-oic acid

Figure 1 Structure of 6α, 7β-dihydroxyvouacapan-17β-oic acid.

in *P. emarginatus* or *P. polygalaeflorus* seeds, respectively, was previously found to be associated with an anti-inflammatory activity in these species (Nunan et al 1982). As demonstrated by Nunan et al (1982), the two substances present in *P. polygalaeflorus* seed oil exhibited opposite effects in the carrageenan-induced edema: the 6α-acetoxy-vouacapan-17,7β-lactone, at a dose of 250 μmol kg^{-1} p.o., enhanced the edema by 43%, while 6α,7β-dihydroxyvouacapan-17β-oic acid, in the same dose and route, inhibited the edema by 38%. Although this is indirect evidence that 6,7-dihydroxyvouacapan-17-oic acid is associated with the anti-inflammatory activity of PF1.1, further fractionation is needed to confirm the activity of this derivative and to search if other substances contribute to this activity in this fraction.

Our results clearly show that compounds other than vouacapan derivatives that are present in sub-fractions PF1.2 (geranylgeraniol) and PF1.3 (farnesol) also contribute to the anti-edematogenic activity of OEP. Although PF1.2 exhibits a topical anti-edematogenic effect, it lacks this effect when administered i.p. in the carrageenan model. Curiously, geranylgeraniol related substances (14,15-epoxygeranylgeraniol and 14,15-dihydro-14,15-dihydroxygeranylgeraniol), previously described by Mors et al (1967) in *P. pubescens* fruit oil, have been associated with protective action against the penetration of *Schistosoma mansoni* cercariae when pure or diluted oil of *P. pubescens* was applied topically to the tails of mice (Austin & Frappaolo 1973; Maleki et al 2001). Although this observation could be interpreted as a physicochemical barrier exerted by the geranylgeraniol derivatives to cercariae penetration, it could also reinforce the view that geranylgeraniol derivatives impair the developing inflammatory process required for cercariae penetration (Ramaswamy et al 2000; Angeli et al 2001).

Post-translational prenylation with geranylgeraniol and farnesol is a key event for protein–protein interactions and membrane-associated protein trafficking, many of the prenylated proteins particularly playing roles in signal transduction pathways (Sinensky et al 2000). Statins, drugs that impair prenylation, have been shown to increase COX-2 expression and prostacyclin formation by human aortic smooth muscle cells, the effect being reversed with the addition of geranylgeranyl-pyrophosphate (Degraeve et al 2001). Using primary cultures of endothelial cells, statins have been reported to inhibit the COX-2, interleukin-1beta, interleukin-6 and nicotine adenine dinucleotide phosphate oxidase mRNA and protein expressions, in the latter case the inhibition being reversed by mevalonate, geranylgeraniol, farnesol or cholesterol addition (Inoue et al 2000). The peroxisome proliferator-activated receptors (PPARs) are promiscuous nuclear lipidic receptors that have been implicated in the control of many processes, including inflammation and immunity. Statins have also been shown to be able to induce PPARalpha and PPARgamma mRNA and their protein levels in primary cultured endothelial cells and hepatocytes (Inoue et al 2000). We could therefore speculate that administration of the isoprenoids present in OEP could, in adequate amounts, modulate some cellular mechanisms involved in acute or chronic inflammation. In this context, we must keep in mind that opposite cellular responses can be obtained, depending on the level of prenylation (Weis et al 2002), a fact that could explain a pro-arthritic, rather than an anti-arthritic, effect in the CIA-model, when sucupira doses were increased (Sabino et al 1999a).

Conclusions

In summary, we can conclude from the present study that the oleaginous extract obtained from *P. pubescens* seeds exhibits acute and topic anti-edematogenic activity. We have also demonstrated that compounds other than vouacapan derivatives, such as geranylgeraniol, contribute to the anti-inflammatory activity of OEP. Studies are in progress to identify the components of OEP better with anti-edematogenic and/or anti-inflammatory activity and elucidate their mechanism(s) of action.

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