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Involvement of the nitric oxide/soluble guanylate cyclase pathway in the anti-oedematogenic action of *Pfaffia glomerata* (Spreng) Pedersen in mice

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Abstract

Pfaffia glomerata is used in southern American countries against inflammatory diseases. We have explored the ability of a crude hydroalcoholic extract of *P. glomerata* root (HEPG) to prevent the oedematogenic action of several inflammatory agents in mice. We have examined also the duration of its effects and the mechanisms involved. The oral or intraperitoneal treatment of mice with HEPG (1, 10, 30, 100 or 300 mg kg^{-1}) reduced, in a dose-dependent manner, carrageenan-induced paw oedema in the early (1–4 h) and late (48 h) periods. In the early period, the ID50 value (the median dose that caused 50% inhibition) of HEPG was 60.5 (28.5–128.71) and 20.4 (14.8–28.3) mg kg^{-1} after oral and intraperitoneal administration, respectively. This effect was still evident when HEPG was administered up to 6 h before carrageenan. HEPG inhibited also paw oedema induced by histamine, serotonin, bradykinin, substance P and bacterial lipopolysaccharide. In addition, oral administration of HEPG increased the levels of nitrate and nitrite in the blood of mice. Further, its anti-oedematogenic action against carrageenan was prevented fully by N^G nitro-L-arginine-methyl-ester (10 mg kg^{-1}, s.c.), as well as by methylene blue (20 mg kg^{-1}, s.c.) or 1H-[1,2,4]oxadiazolo[4,3-alpha]quinoxalin-1-one (2 mg kg^{-1}, s.c.). The results indicated that stimulation of endogenous production of nitric oxide, followed by soluble guanylate cyclase activation, was implicated in the anti-oedematogenic action of HEPG.

Introduction

Preparations of the roots of *Pfaffia glomerata* (Spreng) Pedersen, Amaranthaceae, are commonly used in southern American countries against a number of distinct pathological conditions, such as gastritis, diabetes, lapses of memory, stress and inflammatory disorders (De Oliveira 1986; Bruneton 1995). The wide use of this plant in folk medicine can explain why it is popularly known as Brazilian ginseng (an empirical comparison with the Asian ginseng species, *Panax* spp., Araliaceae) and "paratudo" (which means useful for everything).

Although glomeric acid (a triterpenoid), pfameric acid (a nortriterpenoid), ecdysterone, rubrosterone, oleanolic acid and β-glucopyranosil oleanolate have been found in the roots of *P. glomerata* (Shiobara et al 1993), few studies have been performed to investigate its pharmacological properties. *P. glomerata* root extract has been described as an acute amnesic agent in adult rats (de-Paris et al 2000), and as a stimulant for learning and memory in chronically-treated old mice (Marques et al 2004). Freitas et al (2004) showed it to be a protective agent against ethanol- and stress-induced gastric mucosal lesions. In addition, recent findings attributed a weak cytotoxic activity to *P. glomerata* against *Leishmania braziliensis* (Neto et al 2004) and described its ability to decrease carrageenan-induced rat paw oedema and acetic acid-induced abdominal constrictions in mice (Neto et al 2005).

Nevertheless, the range of effects of *P. glomerata* on the induction of oedema is yet to be fully explored. In this work, we have investigated the ability of the hydroalcoholic extract of *P. glomerata* root in preventing the oedematogenic activity of several inflammatory agents, as well as the duration of its effects on carrageenan-induced paw oedema

in mice. In addition, we have provided evidence that nitric oxide production was crucial for the development of the anti-odematogenic properties of *P. glomerata*.

Materials and Methods

Plant material, phytochemical screening, preparation of the extract and high performance liquid chromatography (HPLC) analyses

The starting material was provided by Empresa Catarinense de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI, Itajaí, SC, Brazil). *P. glomerata* was cultivated in Itajaí (SC, Brazil), at the EPAGRI station and identified by Cynthia Hering Rinnert at the Departament of Pharmacy of our University. Voucher specimens were deposited at the Herbarium of the Universidade da Região de Joinville (number 208). The roots were harvested from 1.5-year-old plants and sliced into small pieces. These pieces of root were dried in a circulating air stove until the humidity content was less than 14%, and then they were ground.

The *P. glomerata* root was analysed accordingly to standard methodology (Harbone 1998; Costa 2001) for the presence of alkaloids (by means of Dragendorff, Mayer, Wagner and Bertrand tests), flavonoids ($FeCl_3$ reaction, Shinoda test, Taubock's reaction, NaOH and $AlCl_3$), steroids (Liebermann-Burchard and Salkowsky reactions), anthraquinones (Bornträger reaction), tannins (Gelatin, $FeCl_3$, lead acetate, Vanillin-HCl and HNO_2 tests; Stiasny reaction), coumarins (NaOH/HCl/Ultraviolet test), cardiotonic glycosides (Keller-Kiliani, Kedde and Baljet reactions) and saponins (Froth test).

The drug was extracted at room temperature with aqueous ethanol solution (6:4, v/v) by maceration for eight days, in the proportion drug:extractive liquid of 1:20, w/v. Ethanol was removed by distillation under reduced pressure at less than 60°C, and the extract solution was frozen and stored under light protection. The crude hydroalcoholic extract solution of *P. glomerata* roots (HEPG) presented a dry residue content of $17.39 \pm 0.06\%$ (CV = 0.39%) (w/w).

Samples of HEPG (30 μL) were complexed with 0.2% cobalt chloride solution and sulfuric acid (modified from Vigo et al (2003), and filtered using a 0.2-μm membrane. The samples were analysed using an Hitachi Merck L7200 reversed-phase analytical HPLC system coupled with a UV detector, using a C_{18} analytical column (5 μm, 12.5 cm × 4.6 mm i.d.; Merk) eluted at 1 mL min^{-1}, at 45°C, with a linear gradient from 90% methanol in water to 100% methanol over 30 min. Chromatographic separations were monitored at 284 nm. Total saponin was used as a reference.

Animals

Male Swiss mice (20–30 g) were purchased from Instituto de Tecnologia do Paraná (TECPAR, PR, Brazil) and maintained at our University. Food and water were freely available. The animals were kept under a 12-h light:12-h dark schedule with a controlled temperature (22 ± 1°C). All procedures described here were in accordance with the National Institutes of Health Animal Care Guidelines (National Academy of Sciences 1996).

Drugs

Indometacin, N^G nitro-L-arginine-methyl-ester (L-NAME), methylene blue, 1H-[1,2,4]oxadiazolo[4,3-alpha]quinoxalin-1-one (ODQ), histamine, substance P, serotonin, bradykinin and lipopolysaccharide (LPS, from *Escherichia coli* serotype 0111-B4) were all obtained from Sigma (St Louis, MO, USA). All other reagents were of the highest grade. Indometacin was dissolved in sodium bicarbonate (0.5%). All other drugs, including HEPG, were freshly prepared in bi-distilled water or sterile isotonic saline, when appropriate.

Induction and assessment of paw oedema

The animals were carefully immobilized and received an intradermal injection of carrageenan (300 μg/paw), bradykinin (3 nmol/paw), substance P (SP, 10 nmol/paw), serotonin (5-HT, 10 nmol/paw), histamine (100 nmol/paw) or bacterial lipopolysaccharide (LPS, 10 μg/paw) in the right hindpaw at a maximal volume of 50 μL. The doses of the inflammatory agents were taken from the literature (Campos & Calixto 1995; Fernandes et al 2002). For control parameters the same volume of sterile saline (0.9%) was injected into the contralateral (left) paw. The development of paw oedema was measured by plethysmometry (Ferreira 1979), at 5, 10, 15, 30, 60 and 120 min (for bradykinin, substance P, 5-HT, histamine and LPS) or 1, 2, 3, 4 and 48 h (for carrageenan) after the intraplantar injection of the inflammatory agents. In the experiments with bradykinin, animals were pretreated with captopril (5 mg kg^{-1}, s. c.) 1 h before to prevent the action of kininases (Campos & Calixto 1995).

Treatment with HEPG

Mice were subjected to a single treatment of HEPG (at 1, 10, 30, 100 or 300 mg kg^{-1}) either orally or intraperitoneally, 1 h before the intraplantar injection and measurement of paw oedema induced by phlogogens, as previously described. The control groups received water (0.1 mL/10 g, p.o.) or sterile isotonic saline (0.1 mL/10 g, i.p.), when appropriate. For a more precise evaluation of the effectiveness of HEPG in preventing the induction of oedema, different groups of animals received either 30 or 300 mg kg^{-1} HEPG (p.o., given once) 6 or 12 h before the intraplantar injection of carrageenan. Experiments using indometacin-treated animals (5 mg kg^{-1}, p.o.; administered 1 h before the inflammatory agents) were performed for comparison.

Evaluation of the influence of nitric oxide synthase and guanylate cyclase inhibition on the effects of HEPG

Animals were treated subcutaneously with either L-NAME (10 mg kg^{-1}) or sterile saline (0.1 mL/10 g; control

groups) twice a day (each 12 h) for two consecutive days. On the third day, the same subcutaneous dose of L-NAME or saline was given twice at 4 h and 20 min before the oral administration of HEPG (300 mg kg^{-1}), indometacin (5 mg kg^{-1}) or water (0.1 mL/10 g; control groups). All groups received an intraplantar injection of carrageenan (300 μg/paw) 1 h later and the development of oedema was measured at 1, 2, 3 and 4 h. The dose and frequency of L-NAME administration was based on our own experience (da Silva-Santos et al 2002) and on the estimated half-life of this substance (Conner et al 2000).

Previous studies have shown the involvement of the guanylate cyclase enzyme in the inhibitory effect of nitric oxide on paw oedema development (Fernandes et al 2002; Fernandes & Assreuy 2004). Therefore, we performed experiments to check the influence of methylene blue (20 mg kg^{-1}, s.c.) and ODQ (2 mg kg^{-1}, s.c.) on the anti-oedematogenic action of HEPG. Control animals received sterile saline (0.1 mL/10 g, s.c.). Methylene blue, ODQ or saline were administered at 4 h and at 20 min before the administration of either HEPG (300 mg kg^{-1}, p.o.) or water (0.1 mL/10 g, p.o.). All groups received an intraplantar injection of carrageenan (300 μg/paw) 1 h later, and the development of paw oedema was measured.

Measurement of nitrate and nitrite levels in HEPG-treated animals

To investigate if HEPG treatment was able to increase the endogenous production of nitric oxide, blood samples were collected from mice treated 1 h before with water (0.1 mL/10 g, p.o.) or HEPG (300 mg kg^{-1}, p.o.), with or without pre-treatment with L-NAME (300 mg kg^{-1}, given twice a day for two consecutive days with two additional doses on the third day). Production of nitric oxide was verified indirectly by means of the Griess reaction (Granger et al 1990). Briefly, zinc sulfate-deproteinized plasma samples were subjected to nitrate conversion. Nitrate was converted to nitrite using *E. coli* nitrate reductase for 2 h at 37°C. Samples were centrifuged to remove the bacteria and 100 μL of each sample was mixed with Griess reagent (1% sulfanilamide in 10% phosphoric acid/0.1% naphtyl-ethylenediamine in Milli-Q water) in a 96-well plate and read at 540 nm in a plate reader. Standard curves of nitrite and nitrate (0–150 μM) were run simultaneously. As under these conditions nitrate conversion was always greater than 90%, no corrections were made. Values were expressed as μM NOx (nitrate + nitrite).

Statistical analysis

The results are expressed as mean ± s.e.m. of 6–12 animals per group. Statistical significance was determined using one-way analysis of variance followed by the post-hoc Bonferroni test. Values of $P < 0.05$ were considered statistically significant. The graphs were edited and statistical analyses were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Phytochemical screening and HPLC

The initial phytochemical evaluation showed the presence of anthraquinones (Bornträger reaction) and saponins (Froth test) in the drug (*P. glomerata* root) used to prepare the HEPG (data not shown). In addition, experiments using HPLC revealed closed profiles between chromatograms obtained from HEPG and reference saponin samples (Figure 1A and B, respectively).

Effects of HEPG on mice paw oedema

A single administration of HEPG (1, 10, 30, 100 or 300 mg kg^{-1}), either orally (Figure 1C and E) or intraperitoneally (Figure 1D and F), caused a marked and dose-dependent reduction of paw oedema measured at the first (1–4 h; Figure 1C and D), as well as at the later (48 h; Figure 1E and F) period after carrageenan injection. The calculated ID50 value (the median dose that caused 50% inhibition) for the fourth hour after carrageenan injection (with their respective confidence limits) were 60.5 (28.5–128.71) and 20.4 (14.8–28.3) mg kg^{-1}, after oral and intraperitoneal administration of HEPG, respectively. In addition, this anti-oedematogenic activity was still evident, although less intense, even when HEPG (30 or 300 mg kg^{-1}, p.o.) administration was performed up to 6 h before the intraplantar injection of carrageenan (Figure 2A and B, respectively).

HEPG also generated a strong and dose-dependent reduction in bradykinin- and substance P-induced paw oedema at all evaluated times (5–120 min). In fact, the highest dose used (300 mg kg^{-1}, p.o.) prevented fully the oedema seen 120 min after the injection of these two inflammatory agents (Table 1). The oral administration of 300 mg kg^{-1} HEPG was enough to significantly reduce the initial paw oedema induced by histamine (Figure 3A) and the entire profile of 5-HT- and LPS-induced paw oedema (Figure 3B and C, respectively).

Involvement of the nitric oxide–guanylate cyclase pathway in the anti-oedematogenic effect of HEPG

Treatment with L-NAME did not change the development of paw oedema in saline-treated animals, although it did eliminate the inhibition of paw oedema elicited by HEPG (Figure 4A), but not by indometacin (Figure 4B). Interestingly, we found increased levels of nitrate and nitrate in blood samples of HEPG-treated animals 1 h after its administration, an event that was, as the anti-oedematogenic action of HEPG, avoided by L-NAME (Figure 4C). In addition, generation of paw

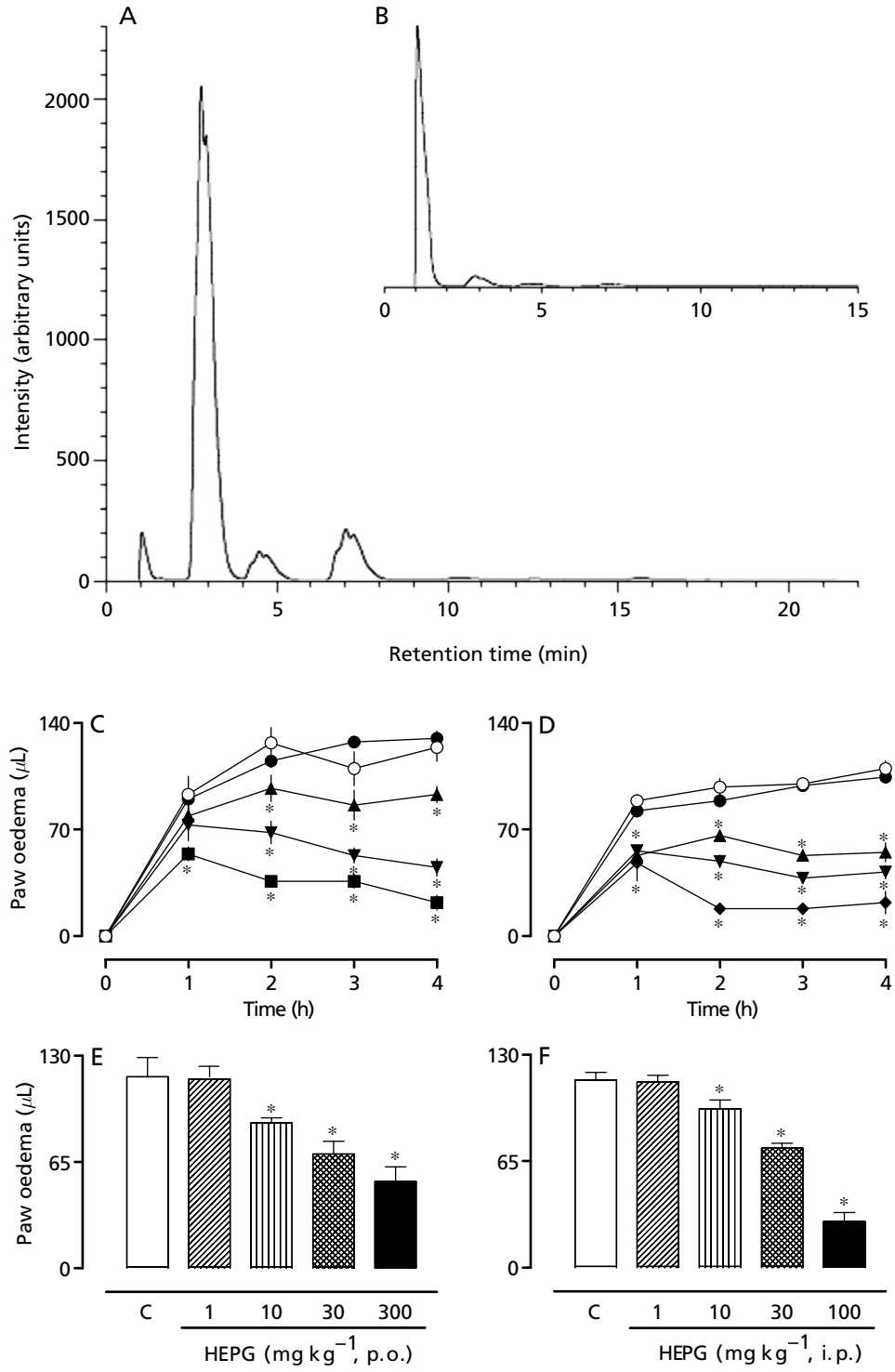

Figure 1 Chromatogram and dose-dependent inhibition of carrageenan-induced mouse paw oedema by HEPG. HPLC of HEPG (A) and reference saponin (B). Influence of either oral (C) or intraperitoneal (D) administration of HEPG at 1 (closed circles), 10 (triangles), 30 (inverted triangles), 100 (diamonds) or 300 (squares) mg kg^{-1}, in the first 4 h of paw oedema induced by the intraplantar injection of carrageenan (300 μg/paw). E and F show the paw oedema measured in the same animals presented in panels B and C, respectively, 48 h after the intraplantar injection of carrageenan. Control animals (open circles or bars) received water (p.o.) or sterile saline (s.c.) 0.1 mL/10 g. All treatments were given 1 h before carrageenan injection. Results are expressed as mean ± s.e.m. (n = 6–10). Where there is no error bar, it is covered by the symbol. Statistical comparison was performed using the one-way analysis of variance followed by *t*-test subjected to the Bonferroni correction. *$P < 0.05$ when compared with the control group.

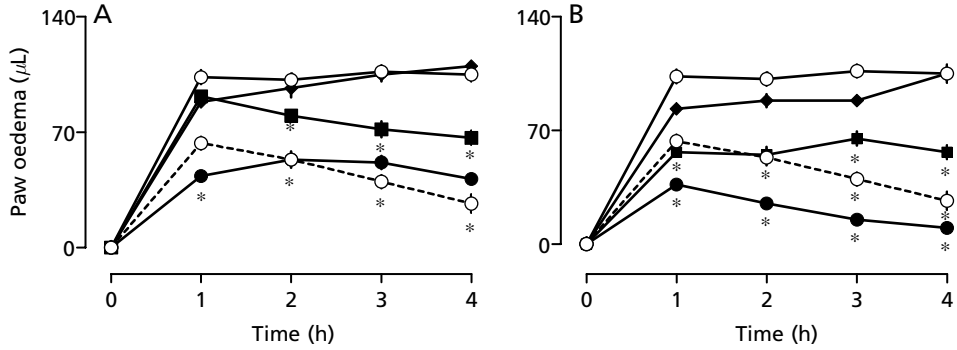

Figure 2 Duration of the effects of HEPG on carrageenan-induced mouse paw oedema. Animals received a single oral administration of HEPG at 30 (A) or 300 (B) mg kg^{-1} at 1 (closed circles), 6 (squares) or 12 (diamonds) h before the intraplantar injection of carrageenan (300 μg/paw). The paw oedema measured in indometacin-(5 mg kg^{-1}, p.o.) treated animals, given 1 h before carrageenan is shown for comparison (open circles with dotted line). For the sake of convenience and since no differences were found between the control groups, only animals that received water (0.1 mL/10 g, p.o.) 1 h before carrageenan were presented as control (open circles with solid line). Where there is no error bar, it is covered by the symbol. Results are expressed as mean ± s.e.m. (n = 6–8). Statistical comparison was performed using the analysis of variance followed by *t*-test subjected to the Bonferroni correction. *$P < 0.05$ when compared with the control group.

Table 1 Dose-dependent inhibition of bradykinin- and substance P-induced mouse paw oedema elicited by a single oral administration of HEPG (30, 100 or 300 mg kg^{-1}) given 1 h before the inflammatory agents

Treatments (p.o.)	Bradykinin-induced paw oedema (10 nmol/paw)				Substance P-induced paw oedema (10 nmol/paw)			
	Time after intraplantar injection (min)				Time after intraplantar injection (min)			
	10	30	60	120	10	30	60	120
Water (0.1 mL 10 g^{-1})	60.8 ± 3.7	41.2 ± 8.1	32.1 ± 7.4	27.1 ± 6.1	26.6 ± 2.6	56.7 ± 3.7	38.3 ± 4	36.7 ± 3.3
HEPG (30 mg kg^{-1})	48.1 ± 4.6	31.8 ± 2.4	27.3 ± 2.4	24.5 ± 2.1	2.8 ± 2.8*	36.7 ± 4.5*	56.7 ± 4.9	45 ± 3.9
HEPG (100 mg kg^{-1})	24.5 ± 4*	23.4 ± 4.8*	19 ± 2.8*	11 ± 2.8*	2.8 ± 2.8*	15.6 ± 4.4*	11.7 ± 4.1*	6.7 ± 2.1*
HEPG (300 mg kg^{-1})	16.9 ± 3.7*	6.3 ± 1.9*	2.7 ± 1.4*	0 ± 0*	1.3 ± 1.3*	4 ± 1.6*	0 ± 0*	0 ± 0*
ID50 (mg kg^{-1})[a]	124.2 (72.7–212.1)	87.3 (46.3–164.8)	125.1 (76.7–203.9)	85.6 (45.2–162)	n.c.[b]	92.1 (58.9–143.9)	152.3 (116.5–199)	117.8 (78.9–190.3)

[a]ID50 with their respective 95% confidence limits. [b]Not calculated (due to the wide inhibition of oedema even at lower doses). Each value represents the mean ± s.e.m. (6–14 animals) of the increase in paw volume. Statistical analyses were performed by means of the analysis of variance followed by *t*-test subjected to Bonferroni's correction. *$P < 0.05$ when compared with water-treated animals at the same point.

oedema was unchanged by HEPG in animals treated with either methylene blue (Figure 5A) or ODQ (Figure 5B).

Discussion

The results confirmed the anti-oedematogenic ability of the crude hydroalcoholic extract of *P. glomerata* root (HEPG), recently described by Neto et al (2005). The results disclosed the nitric oxide–guanylate cyclase pathway as a crucial mechanism involved in the anti-inflammatory effect of HEPG. This has reinforced the value of this plant as a phytomedicine, as well as the importance of new approaches to providing a clearer understanding regarding its pharmacological effects.

The first 2 h of rat paw oedema generated by carrageenan are histamine-, serotonin- and bradykinin-dependent, whereas the following hours (2–4) have been attributed to a rise in prostaglandin production (Di Rosa et al 1971; Conner et al 2000) and neutrophil migration (Di Rosa & Sorrentino 1968). The same mediators and events are putatively involved in mouse paw oedema elicited by carrageenan (Sugishita et al 1981). Thus, the final paw oedema generated by carrageenan is the result of a complex relationship between several mediators, and our results disclosed that the previous treatment of mice with HEPG reduced not only carrageenan-, but also bradykinin-, histamine-, serotonin- and substance P-induced paw oedema. Further, the oedema elicited by LPS, which is commonly responsible for inflammatory responses generated

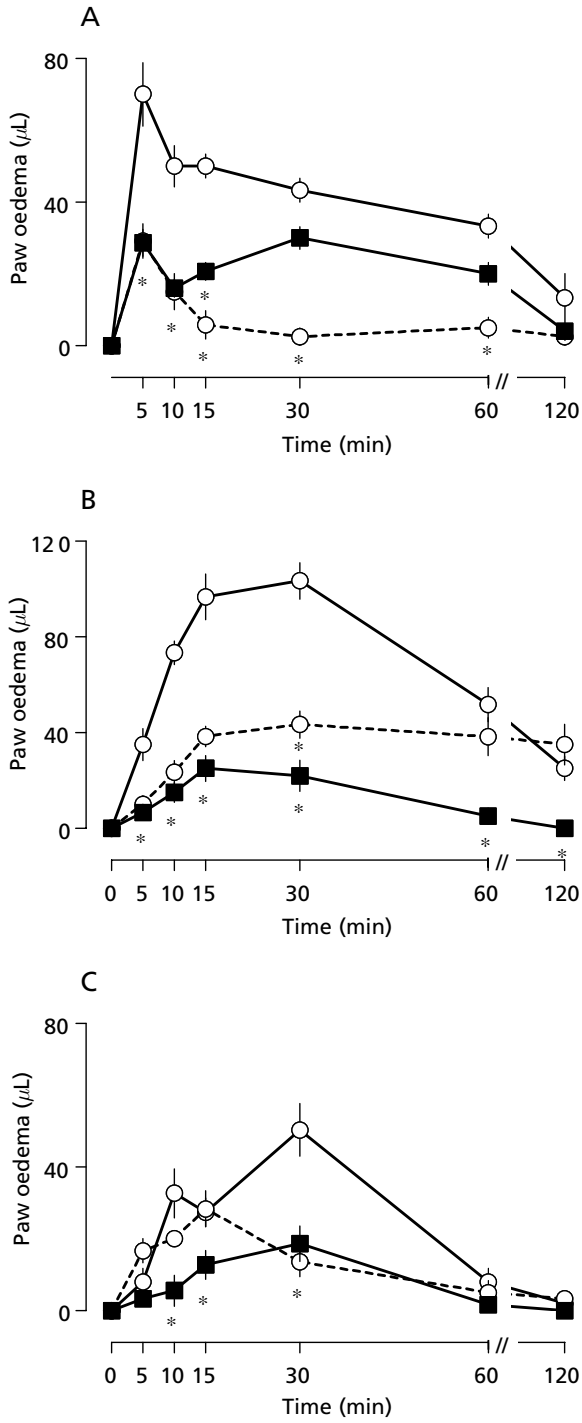

Figure 3 Effects of HEPG on mouse paw oedema induced by different inflammatory stimuli. Animals were pretreated with HEPG (300 mg kg^{-1}; squares), indometacin (5 mg kg^{-1}; open circles with dotted line) or water (0.1 mL kg^{-1}; open circles with solid line) orally 1 h before the intraplantar injection of (A) histamine (100 nmol/paw), (B) serotonin (10 nmol/paw) or (C) LPS (10 μg/paw). Results are expressed as mean ± s.e.m. (n = 6). Where there is no error bar, it is covered by the symbol. Statistical comparison was performed using the analysis of variance followed by *t*-test subjected to the Bonferroni correction. *$P < 0.05$ when compared with the control group.

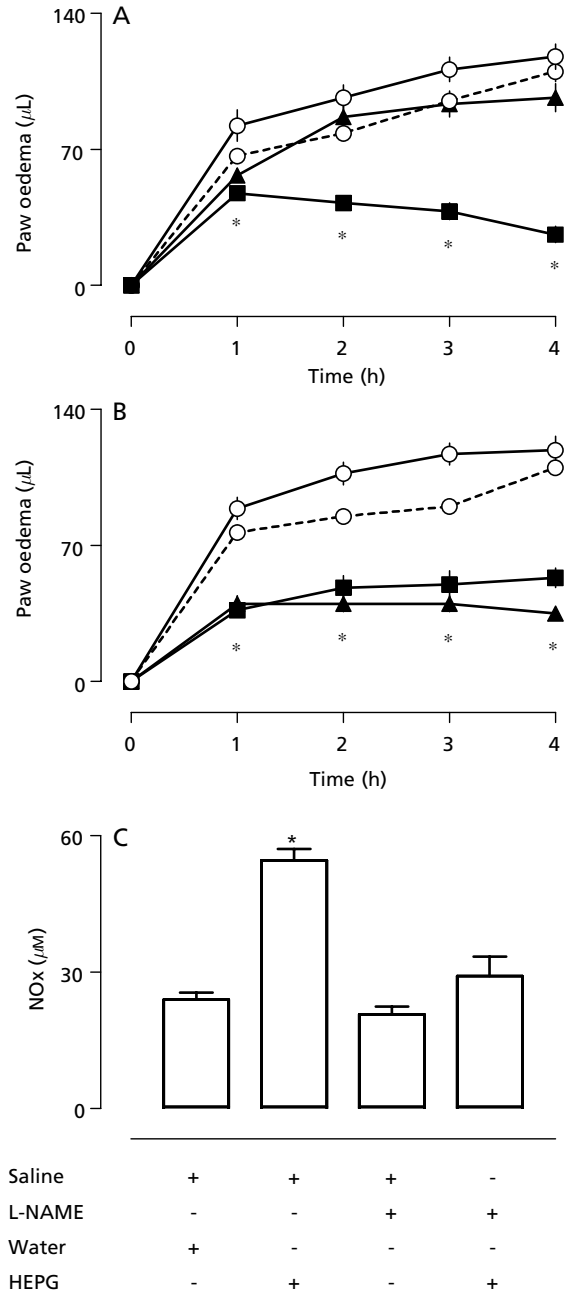

Figure 4 Role of nitric oxide in the anti-oedematogenic action of HEPG. Paw oedema was measured in mice that received either (A) HEPG (300 mg kg^{-1}, p.o.) or (B) indometacin (5 mg kg^{-1}, p.o.) 1 h before the intraplantar injection of carrageenan (300 μg/paw) and were subjected to a repeated treatment (for details see Methods) with either sterile saline (0.1 mL/10 g, s.c.; squares) or L-NAME (10 mg kg^{-1}, s.c.; triangles). Open circles with dotted line show the paw oedema measured in L-NAME-treated animals that received water (0.1 mL/10 g, p.o.) instead of HEPG. Open circles with solid line show the control group (treated with saline instead of L-NAME and water instead of HEPG). The plasmatic level of nitrate + nitrite was measured by means of the Griess reaction in animals subjected to the same treatments (C). Results are expressed as mean ± s.e.m. (n = 8–10). Where there is no error bar, it is covered by the symbol. Statistical comparison was performed using the analysis of variance followed by *t*-test subjected to the Bonferroni correction. *$P < 0.05$ when compared with the control group.

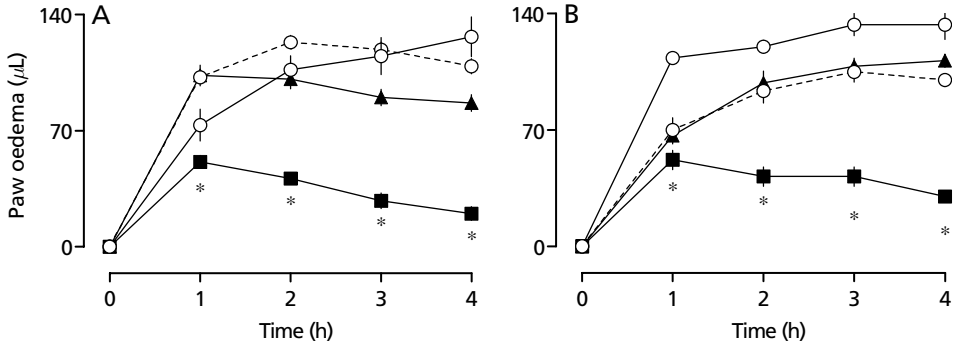

Figure 5 Prevention of the effects of HEPG on carrageenan-induced mouse paw oedema by soluble guanylate cyclase inhibitors. Anti-oedematogenic effects of HEPG (300 mg kg^{-1}, p.o.) administered 1 h before the intraplantar injection of carrageenan (300 μg/paw) in animals treated with sterile saline (0.1 mL/10 g, s.c.; squares) or with either (A) methylene blue (20 mg kg^{-1}, s.c.; triangles) or (B) ODQ (2 mg kg^{-1}, s.c.; triangles), administered twice, at 4 h and at 20 min before HEPG. In A and B open circles with dotted line show the paw oedema measured in methylene blue- or ODQ-treated animals that received water (0.1 mL/10 g, p.o.) instead of HEPG. Open circles with solid line show the control group (treated with saline instead of guanylate cyclase inhibitors and water instead of HEPG). Results are expressed as mean ± s.e.m. (n = 8–12). Where there is no error bar, it is covered by the symbol. Statistical comparison was performed using the analysis of variance followed by *t*-test subjected to the Bonferroni correction. *$P < 0.05$ when compared with the control group.

by Gram-negative bacteria in-vivo, was strongly reduced by HEPG (Figure 3C). Interestingly, LPS-induced paw oedema involves the expression of pro-inflammatory cytokines that are not stimulated by carrageenan, such as tumour necrosis factor-α and interleukin-6 (Vajja et al 2004). This wide anti-oedematogenic action of HEPG is strongly indicative that the mechanisms involved in its effects are probably related to some event capable of preventing the generation of oedema, independent of the stimulus, rather than any specific action, such as blocking membrane receptors.

The anti-oedematogenic effect of the highest dose of HEPG used in our study (300 mg kg^{-1}) was similar to the effects of a standard dose of indometacin (5 mg kg^{-1}), a classical cyclooxygenase inhibitor (dotted lines in Figures 2 and 3). However, while one of the most important side effects of indometacin is the development of gastric disturbances, *P. glomerata* is widely used in popular medicine to treat gastritis and gastric ulcers. Further, it has been shown that a crude aqueous extract of *P. glomerata* root can protect rats against ethanol- and stress-induced gastric ulcers, an action related, at least in part, to the ability of this extract to improve the endogenous production of nitric oxide (Freitas et al 2004).

Nitric oxide has been described as either a pro- or anti-inflammatory agent, a role which appears to be related to the experimental model and with its direct or secondary effects in different cells (for a recent review see Tritto & Ambrosio (2004)). Among the evidence that indicates the ability of nitric oxide to decrease the inflammatory process, Fernandes et al (2002) demonstrated that an injection of small amounts of nitric oxide donors could affect the oedematogenic action of several mediators, besides carrageenan, as a long-lasting soluble guanylate cyclase-dependent event. Interestingly, nitrate and nitrite concentration, a parameter widely used as indicative of nitric oxide production, was found to be significantly increased in the blood of animals treated with HEPG (300 mg kg^{-1}, p.o.), an event that, as well as the anti-oedematogenic action of HEPG, was strongly reduced by the non-selective nitric-oxide synthase inhibitor L-NAME (Figure 4A and C). On the other hand, the effects of indometacin remained unchanged in L-NAME-treated mice (Figure 4B). This finding revealed that an increase in endogenous nitric oxide production was a crucial event for the anti-oedematogenic effects of HEPG, but not for indometacin. This suggested that the anti-inflammatory mechanisms of HEPG and non-steroidal agents were, at least in part, unrelated. Furthermore, the efficacy of HEPG was eliminated in animals treated with methylene blue or ODQ (Figure 5A and B, respectively). This clearly indicated that the production of nitric oxide induced by HEPG was followed by activation of the soluble guanylate cyclase enzyme, one of the most important cellular targets of nitric oxide (Ignarro 1991).

It needs to be investigated how HEPG stimulated the endogenous production of nitric oxide and whether its influence on nitric oxide production could explain the reduction of mice paw oedema seen 48 h after the intraplantar injection of carrageenan, that was associated with an increased infiltration of macrophages, eosinophils and predominantly lymphocytes, rather than release of mediators (Henriques et al 1987). Although oedema may only be one event in the complex pathophysiology of inflammation, the ability of HEPG in almost eliminating the oedematogenic effects of bradykinin and substance P (Table 1), two well characterized mediators involved in nociception (for review see Millan (1999)), suggested that this extractive solution was equally active as an antinociceptive agent, as previously demonstrated by Neto et al (2005), using the acetic acid-induced abdominal constriction test. The activation of nitric oxide-dependent pathways has been associated with reduction of nociception (Sachs et al 2004). It needs to be verified if endogenous nitric oxide is equally involved in the antinociceptive activity of HEPG.

Preliminary phytochemical procedures (mainly Froth test) and HPLC analyses disclosed that the extracted solution of *P. glomerata* used in our experiments possessed saponins among its constituents. Saponins, attached to either steroidal or triterpenoid structures, have been described by several authors as the main constituent of *P. glomerata* (Shiobara et al 1993; Vigo et al 2003). These substances occur widely in plant species and exhibit a range of biological properties (for review see Rao & Gurfinkel 2000; Sparg et al 2004). Among these properties, some saponins have been described as able to relax both vascular and non-vascular smooth muscle due to actions as nitric oxide donors (Kim et al 1998), stimulation of endogenous nitric oxide release (Chen & Lee 1995; Lamping & Nuno 1996; Tanner et al 1999) or induction of nitric oxide synthase expression (Kim et al 2003; Zhang et al 2004). Nevertheless, the real role of saponins and their relationship with the anti-inflammatory effect of *P. glomerata*, and characterization of the active compound (or compounds) responsible for this effect require investigation.

Conclusion

While the anti-inflammatory effects of a number of medicinal plants have been attributed for their ability to inhibit nitric oxide release (Kim et al 2000, 2004; Ahn et al 2005), our results showed that the anti-oedematogenic action of the hydroalcoholic extractive solution of *P. glomerata* depended on the stimulation of endogenous nitric oxide production. Although complex mechanisms may be involved in this phenomenon, this finding has contributed to a clearer understanding of the putative dual role of nitric oxide in the inflammatory process. Furthermore, nitric oxide is involved in either control or genesis of several physiological and pathological conditions. Thus, the ability of *P. glomerata* to positively modulate the endogenous production of nitric oxide may help us to understand why this plant is used in popular medicine against a wide variety of unrelated diseases.

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