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Pharmacology of *Piper marginatum* Jacq. a folk medicinal plant used as an analgesic, antiinflammatory and hemostatic

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Summary

The pharmacological activities of the water extract of *Piper marginatum* Jacq. (Piperaceae), a plant reputed in the Brazilian folk medicine for its analgesic/antiinflammatory, hemostatic and skin wound-healing properties, were assessed. Intraperitoneal injection (i. p.) of the extract (0.1 to 1 g/kg) in mice and rats caused piloerection, sialorrhea, lacrimation, muscle relaxation and dyspnea. At doses above 1 g/kg the extract caused respiratory arrest and death. Intravenous injection of the extract (0.1 to 0.5 mg/kg) into anesthetized rats caused a dose-related hypertension (by 27 to 48 %) that was blocked by prazosin (1 mg/kg) and yohimbine (2 mg/kg). Pithing, reserpine treatment and ganglionic blockade with hexamethonium (5 mg/kg) enhanced the effect. Oral treatment of unanesthetized rats and intragastric administration to anesthetized animals also produced hypertension. The sympathomimetic activity of the extract in isolated vas deferens, left atria and mesenteric arterial bed preparations paralleled that of noradrenaline, and was blocked to the same extent as noradrenaline by α-blockers. The plant extract (0.5 and 1 g/kg, p. o.) also reduced carrageenin-induced paw edema in rats by 80 to 90 % of the control, but it had less effect on the volume of exudate and leucocyte migration in carrageenin-induced pleurisy. Likewise, the extract had a small analgesic effect on the acetic acid-induced writhing test in mice.

It is concluded that the antiedema effect of the plant extract is mainly related to its vasoconstrictor constituent(s). This sympathomimetic activity may explain the plant's reputed hemostatic properties when applied topically to bleeding skin wounds. The predominant vasoconstrictor component of *P. marginatum* detected in HPLC analysis was noradrenaline, whose activity is apparently preserved in the crude extract and produces vasoconstriction after oral administration.

Key words: *Piper marginatum* Jacq., hypertension, sympathomimetic activity, antiinflammatory, medicinal plant.

Introduction

Five genera and 700 species of Piper are believed to grow in Brazil. Some of these plants are used in folk medicine particularly *Piper marginatum* Jacq., a bush widely distributed in tropical regions (Hoehne, 1939). Depending on the region it grows, this species is known by different popular names: "malvaisco" in northern Brazil, "caapeba" and wild-pepper in the Amazonian region, and "Ti bombe" in the French Guyana. In Brazil an extract of the leaves prepared in hot water is used to treat toothache, rheumatism, tumors and bleeding skin wounds (Corrêa, 1984).

P. marginatum Jacq. is also popular snake-bite medicine (Pereira, 1992), and is called the "soldier's herb" because of its powerful hemostatic effect (Braga, 1960). The same plant is used in Puerto Rico to reduce menses flow, in Panama to decrease fever and lung secretions, and in French Guyana to treat skin rashes (Morton, 1977).

Recent investigations on *P. marginatum* are scarce: flavonoids and hydroxibenzoic acid were reported in this specimen (Maxwell and Rampersad, 1988; Tillequim et al., 1978), but no pharmacological data were provided. Studies on related species reported the presence of dopamine and delta-aminobutiric acid in *P. amalago* L. (Durand et al., 1962); methysticine, dihydromethysticine, kavaic acid and dihydrokavaine were detected in *P. methysticum* Forst (Buckley et al. 1979; Youngken, 1943); brachyamide A and B and dihydrobrachyamide were found in *P. brachystachyum* (Koul et al. 1988). Depression of the central nervous system, blockage of serotonin action and local anesthetic activities have been attributed to *P. methysticum* (Meyer, 1979); both hypertension and decreased blood pressure have been attributed to principles isolated from *P. amalago* L. (Durand et al. 1962); while *P. longum* has been shown to induce paw-edema in rats (Nazar et al. 1970).

Based on the reported folk medicine use of *P. marginatum* Jacq., the present study aimed to evaluate the antialgic/antiinflammatory and other general effects produced by the plant's water extract. The results confirm the antiedema activity of the extract. This effect, however, was related to a general vasoconstriction that is likely to account for the hemostatic activity attributed to the plant. Noradrenaline was identified as the extract's major vasoconstrictor constituent.

Materials and Methods

Plant extract and HPLC analysis. Piper marginatum Jacq., a large 1–2 m bush with heart-shaped, scented leaves and abundant long, thin inflorescences was collected in Recife, State of Pernambuco, in the northeastern of Brazil from June to August 1988. The plant was grown in a Botanical Garden (Horto Botânico de Dois Irmãos) and was identified by Dr. Suzene I. Silva of the Department of Botany, Federal University of Pernambuco, in whose Herbarium a sample was filed under the number 20040 UFP. The vegetal material was dried at 32 °C during 48 h and the leaves were ground. A water extract (WE, 5 %) was prepared according to folk indication by adding the ground plant to boiling water and keeping the mixture at 40 °C for 30 min. After filtration the extract was concentrated under vacuum and freeze-dried (yield = 13.5 % w/v). A preliminary phytochemical screening was performed as described by Marini-Bettolo (1980).

For the HPLC analysis 50 g of the dried leaf powder were treated with 10 % acetic acid at room temperature for 12 h. After repeated washing with distilled water, the acid was removed and the extract dried under vacuum. The material was then stirred for 10 min with 15 g of alumina and 0.5 N Na_2CO_3 at pH 8.0–8.5, then eluted in a glass column with 100 mL of water and 400 mL 0.1 N HCl. The acid samples (pH 2–4) obtained were pooled and treated with 0.5 N Na_2CO_3 to pH 6.5. The precipitate was discarded and the soluble material was filtered through an ion exchange Amberlite IRC-50 (H^+) column eluted with water and 0.67 boric acid (Price and Price, 1957). The acidic eluate (50 µL) was qualitatively analyzed by HPLC in comparison to noradrenaline.

The HPLC system was a LC-6A Shimadzu with an electrochemical detector, Ag-AgCl/glass electrodes, and an operating voltage of +5 V (Guillemin et al. 1988; Murai et al. 1988). The column was a Shim-pack CLC-ODS 15 × 0.6 cm reverse phase. The mobile phase was methanol 10 %; 0.02 M citrate-phosphate buffer pH 2.6, EDTA 0.12 mM, 0.05 % heptanesulfonic acid operating at +0.5 V, flux of 0.7 mL/min, and a pressure of 40 kgf/cm^2. Noradrenaline (NA, 2 to 16 ng/mL) was the internal/external standard to which the samples were compared. Both the retention time, when NA was the external standard, and the increased area under the curve, when NA was the internal standard, were used to confirm the identity of both substances.

General pharmacological screening. Wistar adult rats (180–220 g) and albino mice (25–30 g) of either sex were treated intraperitoneally (i. p.) or orally (p. o.) with the extract (0.01 to 2 g/kg). The animals were observed hourly for 6 h, then after 12 and 24 h. Control animals were injected with the vehicle (saline). Changes in behavior and physical alterations were entered on recording sheets adapted from those described by Malone (1977).

Blood pressure recordings. The mean carotid blood pressure was recorded from rats anesthetized with urethane (0.8 g/kg) + sodium pentobarbital (25 mg/kg) i. p. using a Statham P23 AA transducer and a Beckman polygraph as previously detailed (Carvalho and Lapa, 1990). In some experiments, the blood pressure was recorded from rats pretreated with reserpine (Serpasil – Roche, 10 mg/kg i. p., 24 h and 2 h before the experiments), or pithed animals (Carvalho and Lapa, 1990). Drugs were injected via the external iliac vein in volumes never exceeding 0.3 mL. In a few animals the plant extract was administered through an intragastric cannula introduced under anesthesia.

For blood pressure recordings from conscious rats, a catheter was placed in the carotid artery of animals anesthetized with ether. After 24 h, the catheter was connected to a pressure transducer and the basal arterial blood pressure recorded before oral administration of the extract and up to 3 h afterwards (Weeks and Jones, 1960).

Rat vas deferens. The vas deferens was excised from male rats under light ether anesthesia, then stripped of connective tissue. The organ was suspended in a 10 mL bath con-

taining physiological solution (in mM: NaCl 138; KCl 5; $CaCl_2$ 1.8; NaH_2PO_4 0.36; $NaHCO_3$ 15 and glucose 5.5, pH = 7.6) at 30 °C and isotonic contractions were recorded under 1 g tension. After 30 min stabilization, cumulative concentration-response curves were constructed to noradrenaline (NA; 10^{-8} to 10^{-4} M) or the extract (2 to 512 µg/mL) (van Rossum, 1963) prior to or after 10 min exposure to yohimbine (10^{-5} M), prazosin (10^{-7} M), or imipramine (10^{-7} to 10^{-6} M). The responses of *vasa deferentia* from reserpine-treated rats to tyramine (10^{-7} to 10^{-3} M) were recorded prior to and after incubation of WE or NA.

Guinea-pig atria. Guinea pigs (300–350 g) were stunned and exsanguinated. Their left atria were dissected out and mounted in 10 mL organ baths containing Krebs solution (in mM: NaCl 135; $NaHCO_3$ 15; KCl 5; $CaCl_2$ 2; $MgCl_2$ 1; NaH_2PO_4 1; and glucose 11, pH = 7.4 after gassing with 95 % O_2–5 % CO_2), at 37 °C. The tissues were electrically driven by square pulses (2 ms, 4 Hz, supramaximal voltage) through platinum electrodes immersed in the bath. Isometric contractions were recorded with a force transducer on a Beckman R-411 recorder. After 30 min stabilization, the effects of the extract (2.5 to 10.0 µg/mL) or isoproterenol (0.25 to 1 µM) were recorded in the absence or presence of propranolol (2 µM).

Perfused rat mesenteric arterial bed. The mesenteric vessels were dissected from anesthetized rats as described by MacGregor (1965). The pancreato-duodenal and ileo-cholic branches of the mesenteric artery were ligated and the superior mesenteric artery perfused with 4 mL/min of Krebs at 37 °C. After removal of the jejunum and ileum, the mesenteric preparation was placed in a perfusion chamber. After 30 min stabilization, noradrenaline (1 to 16 µg) or WE (0.1 to 0.8 mg) was injected prior to and after treatment with yohimbine (0.5 µg).

Analgesic activity. Mice were treated with the vehicle (saline) or the extract (0.5 and 1 g/kg, p. o.) 30 min prior to the injection of 0.8 % acetic acid (0.1 ml/10 g body weight) and the number of writhes was counted at 5 min intervals for 30 min (Koster et al. 1959; Freire et al. 1993). Animals treated with indomethacin (10 mg/kg p. o.) were used for positive control.

Antiinflammatory activity. Female rats were treated with either the vehicle (saline), the extract (0.5 and 1 g/kg) or indomethacin (10 mg/kg) p. o. 30 min prior to the injection of 0.1 ml of 1 % carrageenin into the right hind paw. The contralateral paw was injected with an equal volume of saline. The paw volumes were determined hourly by plethysmography (Winter et al., 1962) for 5 h. The paw swelling was calculated as the difference between the two paws as a percentage of the initial volumes (Freire et al., 1993).

Pleurisy induced by carrageenin. Rats were pretreated with the vehicle or the extract (0.5 and 1.0 g/kg) 60 min prior to the intrapleural injection of 0.25 mL of 0.2 % carrageenin in saline into the right pleural cavity. After 3 h the animals were anesthetized with ether and exsanguinated. The pleural exudate was collected and the cavity washed with 1 ml saline containing heparin (10 UI/ml) (Vinegar et al., 1973). The number of leucocytes that had migrated to the pleura was counted in a cell counter (CELM, CC-510) (Freire et al., 1993).

Statistical analysis of data. The data were presented as means ± s.e.m. The EC50 values (mean effective concentration) were presented as geometric means and confidence limits (CL). Differences among two means were determined using the Student "t" test. Differences between more than 2 means were determined using one-way analysis of variance followed by the Tukey-Kramer test (Sokal and Rohlf, 1981). Data were considered different at a significance level of $P < 0.05$.

Results

Plant constituents

The phytochemical screening of *Piper marginatum* Jacq. indicated that steroids/triterpens, flavonoids, cinamic derivatives and noradrenaline were present in the leaves; no cardenolides, tannins, cumarin or alkaloids were detected.

The HPLC analysis of the plant extract obtained after ion exchange chromatography and adsorption in alumina revealed a major peak with a retention time of 9.7 min that was coincident with the retention time of noradrenaline. When the extract and NA were mixed at known amounts prior to injection into the column, the peak at 9.7 min was increased and the area under the peak was equal to the sum of the corresponding area of NA and the extract, indicating the presence of this cathecolamine in *P. marginatum* extract.

General effects

Rats and mice treated orally with up to 2 g/kg of the water extract (WE) did not show signs of toxicity or change in spontaneous motor activity when compared to control animals. Likewise, no significant effects were detected in animals treated i. p. with up to 10 mg/kg of the extract. At high doses (0.10 to 1 g/kg i. p.), the extract produced piloerection, quietness, sialorrhea, lacrimation, ear palor, muscle relaxation, resting of the head on the table and dyspnea. The effects were related to the dose. All the animals treated with the highest doses died within 15 min because of respiratory arrest.

Effects on the arterial blood pressure

The mean blood pressure (BP) of control anesthetized rats was 95.5 ± 1.6 mmHg (n = 8). Intravenous injection of 0.1, 0.25 and 0.5 mg/kg WE produced a proportional increase of BP, respectively, 25.6 ± 4.1; 34.2 ± 3.7 and 46.0 ± 4.0 mmHg. After pithing, the blood pressure was lowered

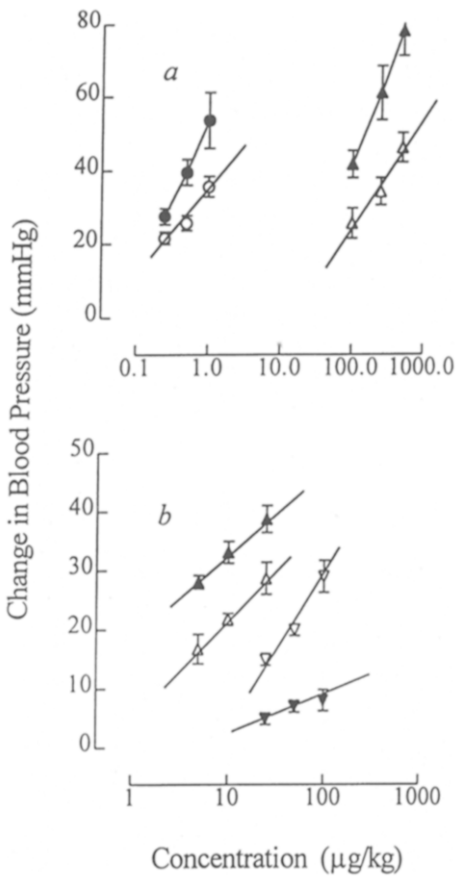

Fig. 1a. Effects of the extract of *Piper marginatum* Jacq (△, ▲) and noradrenaline (○, ●) on the blood pressure of anaesthetized intact (open symbols) or pithed (closed symbols) rats. Symbols and vertical bars are means ± s.e.m. of 6 experiments in each group.
1b. Effect of the extract of *Piper marginatum* Jacq (△, ▲) and tyramine (▽, ▼), on the blood pressure of control anaesthetized rats (open symbols) or reserpine-treated animals (closed symbols). Symbols and vertical bars are means ± s.e.m. of 4 experiments in each group.

to 43.5 ± 3.0 mmHg and the responses to NA were increased by 70 %. The extract was more effective in these same rats, but the dose-response curves obtained in both the intact and pithed rats were parallel (Fig. 1a). Previous treatment with prazosin (1 mg/kg i. v.) or yohimbine (2 mg/kg i. v.) reduced the responses of both WE (0.2 mg/kg) and NA (0.5 μg/kg) by respectively, 35 % and 37 % (n = 4).

Ganglionic blockade of anesthetized rats with hexamethonium (5 mg/kg, i. v.) reduced the basal BP by 26.8 ± 9.0 mmHg. Injections of *Piper* extract (50 μg/kg) or NA (0.25 μg/kg) into these animals increased the blood pressure from 30.5 ± 2.7 to 42.5 ± 2.8 mmHg, and from 29.5 ± 2.7 to 40.5±2.8 mmHg, respectively (n = 4).

Pretreatment of rats with reserpine (20 mg/kg i. p.) caused depression, ptosis, piloerection and diarrhea. Upon anesthesia, the basal BP of these animals was 52.2 ± 6.3

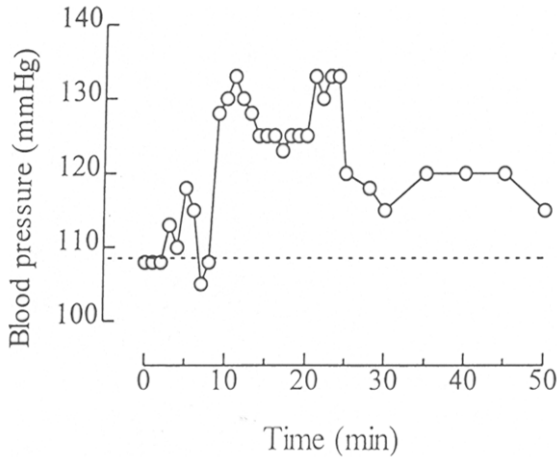

Fig. 2. Effect of oral administration of *Piper marginatum* Jacq extract (1 g/kg) on the blood pressure recorded from unanaesthetized rat: a representative experiment. The dotted line represents control values of blood pressure.

mmHg (n=4). Under this condition, the hypertensive responses to tyramine (25 to 100 μg/kg i. v.) were reduced by 30 % comparatively to those observed in the control rats. In the same animals, the responses induced by the extract (5 to 20 μg/kg i. v.) were increased by 35 to 65 % (Fig. 1*b*).

Effects of oral administration of WE

Administration of WE (1 g/kg) to anesthetized rats through an intragastric cannula produced a progressive hypertension that reached its maximum within 10 min (from 102.0 ± 7.4 mmHg to 126.4 ± 0.6 mmHg, n = 5). The effect lasted 30 min and was reversed to control values after 60 min. Control animals treated with equal volumes of water p. o. did not show significant change in their BP. Previous treatment with yohimbine (2 mg/kg i. v.) reduced the basal BP of these animals by 38.4 ± 7.3 mmHg and reduced the hypertensive effect induced after oral administration of the extract (1 g/kg) by 35 %. Similar effects were obtained after pretreatment with prazosin (1 mg/kg, i. v.).

The mean BP recorded from control unanesthetized rats was around 108 mmHg (n = 3). Oral administration of the vehicle (water, 0.5 ml) did not change the BP. Administration of the extract (1 g/kg, p. o.) to these animals produced hypertension of 20 mmHg which peaked in about 10 min, lasted for 15 min and was restored to control values after 50 min (Fig. 2).

Effect on the rat mesenteric arterial bed

Bolus injection of either WE (0.1 to 0.8 mg) or NA (1 to 16 μg) produced a dose-dependent increase of the perfusion pressure of the rat mesenteric arterial preparation with an EC50 of 159.6 μg (CL: 135.5–187.5) and 2.23 μg (CL: 1.90–2.63), respectively (n = 4). After an injection of

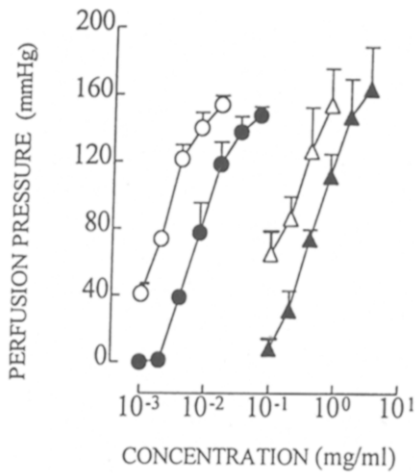

Fig. 3. Concentration-response relationships of the extract of *P. marginatum* Jacq (△, ▲) and noradrenaline (○, ●) on perfusion pressure of the rat mesenteric arterial bed obtained before (open symbols) and after perfusion of yohimbine (0.5 µg/mL) (closed symbols). Symbols and vertical bars are means ± s.e.m. of 4 experiments.

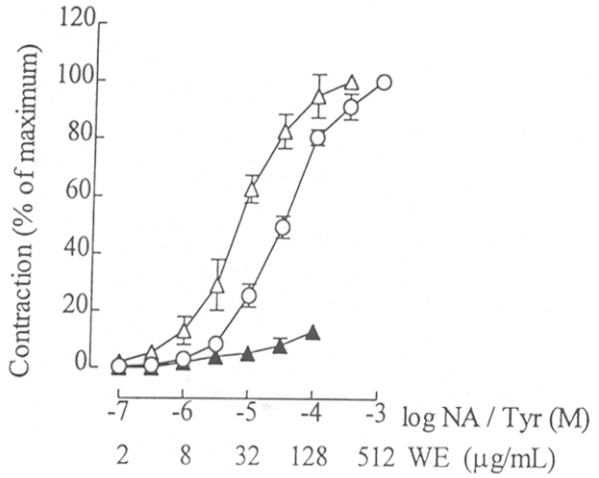

Fig. 4. Concentration-response curves constructed in isolated vas deferens preparations to noradrenaline (NA, ○), the water extract of *Piper marginatum* Jacq (WE, △) and tyramine (Tyr, ▲) after the extract. Symbols and vertical bars are means ± s.e.m. of 5 experiments in each group.

yohimbine (0.5 µg) the concentration-response curves were shifted in parallel to the right, with no significant change of the maximal responses. The EC50 values of WE and NA determined in these preparations were, respectively, 447.53 µg (CL: 406.44–562.34) and 7.78 µg (CL: 6.61–9.14) (Fig. 3).

Effect on the rat vas deferens

The addition of successive and increasing concentrations of WE (2 to 512 µg/ml) or NA (10^{-8} to 10^{-5} M) to the isolated rat vas deferens produced contractions proportional to the concentrations, with EC50 values of 38.02 µg/ml (CL: 29.11–49.77) and 1.69 × 10^{-6} M (1.33–2.15), respectively (n = 5). Incubation of yohimbine (10^{-5} M) caused a parallel and rightward shift of the concentration-response curves and increased the EC50 values by 3.5- and 5-fold, respectively, without altering the maximal contractile effect. Previous incubation of imipramine (10^{-6} M) potentiated the contractile responses of the vas deferens to either the extract or NA, leading to a more prominent effect at low concentrations of both agents. At concentrations producing 25 to 40 % of the maximal contraction the effects of NA and the extract were potentiated by 105 to 130 % and 70 to 85 %, respectively.

In vas deferens preparations of reserpine-treated rats, the maximal contraction induced by tyramine (10^{-7}–10^{-4} M) reached 15 % of control value. Control responses to tyramine were recovered after construction of the concentration-response curve to NA, but were not obtained after that with WE (Fig. 4).

Effect on the electrically paced rat left atria

Addition of 2.5, 5 and 10 µg/ml of WE increased the contractions of the left atria by 32.5 ± 4.5 %; 111.0 ± 15.4 % and 145.2 ± 16.3 %, respectively. The effect was reversed after washing with nutritive solution. Propranolol (2 µM) reduced the effect of the WE concentrations to 15.0 ± 2.1 %; 22.5 ± 3.4 % and 30.4 ± 5.4 %, respectively. At the same concentration, propranolol reduced the inotropic responses to isoproterenol (1 µM) from 133.3 ± 16.1 % to 41.7 ± 10.0 %.

Effect on the rat paw edema induced by carrageenin

Injection of carrageenin induced a progressive swelling of the rat paw that reached a maximum of 37.0 ± 3.6 % above the initial value (1.18±0.08 ml) within 3 h. Previous treatment of the animals with 0.5 and 1 g/kg WE, p. o., inhibited the paw edema to 21.7 ± 1.9 % and 7.1 ± 2.3 % of control, respectively, after 3 h. On the other hand, treatment with indomethacin (10 mg/kg p. o.) inhibited the paw swelling to 5.2 ± 2.0 %, which did not differ from the control paw injected with saline (Fig. 5a).

Pleurisy induced by carrageenin.

Pleural injection of 0.5 mg carrageenin induced the formation of 0.51 ± 0.05 ml of exudate containing 5.53 ± 0.31 × 10^3 leucocytes/mm^3. Treatment with the Piper extract (0.5 and 1 g/kg, p. o.) prior to the carrageenin injection did not affect the exudate volume (0.48 ± 0.06 and 0.49 ± 0.03 ml respectively), or the number of leucocytes that migrated (5.23 ± 0.46 and 5.15 ± 0.21 × 10^3/mm^3, respectively).

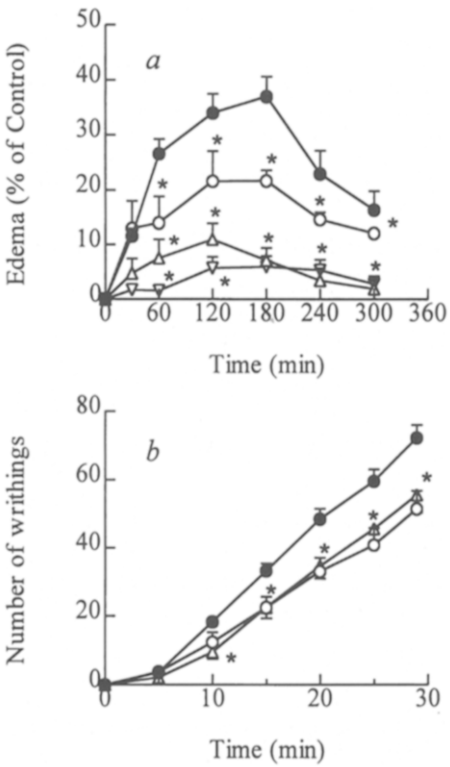

Fig. 5a. Time-course of the paw swelling induced by subplantar injection of 0.1 mL 1 % carrageenin in rats pretreated orally with the vehicle (•), the extract of *P. marginatum* Jacq (0.5 g/kg – ○; 1 g/kg – △), or indomethacin (10 mg/kg – ▽. The differences between the volume of the right paw injected with carrageenin and the left paw injected with saline are plotted in ordinates as percentages of the original volumes at time zero. Symbols and vertical bars are means ± s.e.m. of 5 animals for each group.

Fig. 5b. Accumulative number of writhings induced by intraperitoneal injection of 0.8 % acetic acid (0.1 mL/10 g) in mice after oral administration of either the vehicle (•) or the extract of *Piper marginatum* Jacq (0.5 g/kg – ○; 1 g/kg – △). Symbols and vertical bars are means ± s.e.m. of 8 animals for each group.

Effect on the acetic acid-induced writhing in mice

Treatment of mice with WE (0.5 and 1.0 g/kg p. o.) reduced the number of writhes induced by acetic acid from 72.2 ± 3.7 (control) to 51.4 ± 5.3 and 55.4 ± 5.1 within 30 min, respectively. The differences were statistically significant, but unrelated to the administered dose (Fig. 5b).

Discussion

Traditional medicine in Brazil has attributed to the decoction of *Piper marginatum* Jacq. leaves antiinflammatory, hemostatic and skin healing properties. To validate the plant's medicinal use and to determine the specificity of these actions, pharmacological studies were carried out on mice and rats. *In vitro* studies were undertaken to determine the mechanisms involved in the extract's vasoconstrictor effect as compared to known sympathomimetics agents.

General pharmacological activity tests revealed that at up to 2 g/kg *p. o.*, the crude water extract was relatively non toxic. When administered i. p., however, the extract was toxic at doses above 100 mg/kg, producing autonomic stimulation, muscle relaxation, dyspnea and death caused by respiratory failure. Further *in vitro* experiments were done to verify whether these autonomic effects might interfere with the development of an inflammatory reaction.

In anesthetized rats the plant extract produced a dose-related hypertension that was unrelated to ganglionic stimulation or to neurotransmitter release from sympathetic nerve endings. Blockade of α_1-receptors by either prazosin or yohimbine decreased the pressor responses to both the extract and noradrenaline, indicating that the extract's effect was related to a post-synaptic interaction with adrenoceptors. The hypertensive effect of the extract also occured after oral administration, important evidence for explaining plant's folk use as an antiinflammatory, hemostatic and skin healing medicine.

The *in vitro* tests using rat atria, mesenteric arterial bed and vas deferens confirmed that the extract has sympathomimetic effects resembling those of noradrenaline. In fact, the concentration-response curves to noradrenaline and the extract were parallel, and both were reduced to the same extent by α- or β-blockers. In addition, the blockade of catecholamines uptake by imipramine potentiated the contractile responses of the rat vas deferens to both the extract and noradrenaline, reinforcing the evidence that a sympathomimetic substance is present in the plant.

Further chemical purification and HPLC analysis of the extract provided strong evidence of the presence of noradrenaline as the sympathomimetic principle in *P. marginatum* Jacq. These observations, however, did not explain the sustained hypertension obtained after oral administration of the extract, which was inhibited by α-blockers. In fact, it is known that noradrenaline is inactive when given orally because of its rapid oxidation and conjugation in the gastrointestinal mucosa and liver (Hoffman and Lefkowitz, 1995). Catecholamines were also identified in other plants (Feng et al., 1961; Freire et al., 1993) and dopamine was isolated from other Piper species (Durand et al., 1962). However, none of these plants extracts was active *per os*. The Ephedrine isolated from several Ephedra species is an α- and β-sympathomimetic drug active upon oral administration. Its prolonged action resembles that observed with *P. marginatum* extract, but its main action is exerted indirectly and would not be as active after reserpine treatment as it is in normal rats. On the other hand, the HPLC analysis of the extract using ephedrine, adrenaline, dopamine and noradrenaline as internal standards revealed a peak with a retention time similar only to that of noradrenaline (not shown). This being the case, it is not known what sort of protection is provided

to preserve the catecholamine effects when the extract is administered *per os*.

The changes in blood pressure produced by the extract were apparently unrelated to the validation purposes of this study. It is known, however, that vasoconstriction decreases swelling in an acute inflammatory reaction and that catecholamines counteract the edema produced by phlogistic agents like histamine, serotonin and bradykinin (O'Duffy and Chahl, 1979). Oral administration of the extract in rats produced a hypertensive effect that lasted 50 min and was coincident with the initial phase of the carrageenin-induced paw edema. Because of these concomitant effects, the putative antiinflammatory activity of *P. marginatum* extract could not be disassociated from its general vasoconstriction effect. To clarify this interaction, we investigated the effects of the plant extract on other parameters of the inflammatory process unrelated to the edema. The model chosen was pleurisy induced by carrageenin in rats in which the exudate formed is related to the actions of prostaglandin E2 and some lysosomal enzymes activated by carrageenin, but where the leukocyte migration is unrelated to the activation of cyclooxigenase (Vinegar et al., 1973; Higgs et al., 1980; Mikami et al., 1983; Brooks and Day, 1991). The results showed that while the extract at concentration 1 g/kg p. o. inhibited paw edema by 75 % within 1 h, it did not alter the volume of the pleural exudate, nor did it interfere with the number of leukocytes that migrated to the pleural space.

These results indicated, that the antiedematogenic activity of the extract was most likely due to its vasoconstrictor effect and not to a specific antiinflammatory action of its other constituents. This finding was corroborated by the writhing test in mice in which the mechanism corresponds to an inflammatory reaction induced by prostaglandins synthesized upon injection of irritating agents into the peritoneal cavity (Ferreira and Vane, 1974; Fujiyoshi et al., 1989). In this test the effect of the extract was mild and was unrelated to the dose and to the antiedematogenic effect.

Taken together, the results suggest that the antiedema activity reported after oral administration of the water extract of *Piper marginatum* Jacq. is due to a vasoconstriction action. This action may also explain the reputed hemostatic effect of the plant when applied topically on bleeding skin wounds. Chromatographic analysis of the extract indicates that the vasoactive principle is noradrenaline, which might be somehow protected in the planct extract to produce vasoconstriction when administered *p. o.*

Acknowledegments

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