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Short Communication

Anti-inflammatory effect of *Pimenta racemosa* var. *ozua* and isolation of the triterpene lupeol

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Abstract

Pimenta racemosa var. *ozua* (Myrtaceae) is a tropical plant, used in different inflammatory processes by the folk medicine of the Caribbean region. From the methanol extract of the leaves a terpenic compound identified as lupeol has been isolated for the first time in this species. The anti-inflammatory activity of the extract has been evaluated against two experimental models of acute inflammation: paw edema in rats, using carrageenan or dextran as phlogogen agents, and ear edema in mice, inducing the inflammation with 12-*o*-tetradecanoylphorbol acetate (TPA). Myeloperoxidase activity (MPO) was also assayed as an indicator of leukocytary migration in the inflamed ears. In the carrageenan test, the methanol extract (125 and 250 mg kg^{-1} p.o.) had a dose-dependent and significant effect at different time intervals. On the contrary, when the dextran was injected in paw, the extract did not reduce the inflammation provoked. This behavior was similar to indomethacine (25 mg kg^{-1}) used as a standard drug. In the TPA-induced ear edema, the methanol extract (0.5, 1 and 3 mg ear^{-1}) significantly reduced the inflammation. In the MPO assay a significant inhibition of the enzyme was observed in the inflamed tissue in all the samples assayed. These results show that the methanol extract from the leaves of *Pimenta racemosa* var. *ozua*, is effective against acute inflammation processes, by oral route and when topically applied. The anti-inflammatory behavior of the extract was similar to that exhibited by the selective cyclo-oxygenase inhibitor, indomethacin. On the other hand, the reduction of MPO activity shows that the action mechanism is clearly related with the neutrophil migration.

Keywords: *Pimenta racemosa*; Anti-inflammatory activity; Lupeol; TPA; Carrageenan; Cyclo-oxygenase; Myeloperoxidase

1. Introduction

Pimenta racemosa var. *ozua* (Myrtaceae) is a plant distributed in ''La Española'' island (Dominican Republic) and is known by the popular name of ''ozua'' [1]. This species is used in the folk medicine of the Caribbean basin for different afflictions. In the Dominican Republic, the essential oil extracted from its leaves is used for the local treatment of rheumatism or for toothache. In Cuba the seeds' decoction is used as a stimulant [2]. In Trinidad, it is used for colds and influenza [3]. The leaves' decoction from *P. racemosa* var. *ozua* is used against abdominal pains [4] in Haití.

Some previous pharmacological studies on the species showed that decoctions of the leaves have a spasmolytic effect [4,5], but many of these phytochemical or pharmacological aspects are not well known.

We have extracted the leaves of *P. racemosa* var. *ozua* with different solvents and a preliminary screening showed that the methanol extract is rich in terpenoids, which have always attracted attention and their pharmacological activities have often been evaluated. In the present work the composition of the methanol extract was investigated and a pure compound was isolated and identified as lupeol, a triterpene that has been reported as having anti-phlogistic properties [6,7]. For these reasons we have evaluated the anti-inflammatory activity of the methanol extract on different acute inflammation experimental models: paw edema in rats, where carrageenan or dextran were used as phlogogen

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agents, and ear edema in mice, inducing the inflammation with 12-*o*-tetradecanoylphorbol acetate (TPA) or AA. To determine the possible influence of the extract and the compound on leukocytary migration, myeloperoxidase activity was also measured.

2. Material and methods

2.1. Plant material

The leaves of *Pimenta. racemosa* var. *ozua* were collected at The Natural Park of "Los Haitises" in Santo Domingo (Dominican Republic) in May 1998. The authenticity of this material was confirmed by the National Botanic Garden of Santo Domingo (JBSD)

2.2. Extraction and isolation

Plant material (100 g) was exhaustively extracted with methanol in a soxhlet apparatus. The extract obtained was concentrated under reduced pressure using a rotatory evaporator, yielding a dry residue (26.44%). This residue (5 g) was chromatographed on a silica gel column (90 g, 0.063–0.200 mm and 0.25–0.5 mm, E. Merck, Darmstadt) and eluted with solvent portions of increasing polarity of a mixture of *n*-hexane, ethyl acetate and methanol, yielding 304 fractions (15 ml). Fractions showing identical behavior in thin layer chromatography were combined. Fraction 15, corresponding to the *n*-hexane/ethyl acetate (90:10 v/v) eluate, yielded a crystalline compound (4.8 mg). TLC silica gel developed with *n*-hexane–ethyl acetate (70:30 v/v) gave an orange-brown spot with the oleum reagent (RF 0.59); m.p. 215–216°C; UV λ_{max} (nm) (MeOH) 271sh, 220sh, 205 nm. MS; *m*/*z* (%) 426 [M +] (80), 408 (15), 365 (15), 218 (100), 208 (50), 189 (70), 135 (65), 107 (858), 95 (68). The compound was identified as lupeol, after comparison with reported ^{1}H and ^{13}C NMR spectral data [8,9].

2.3. Animals

Experiments were performed on male Wistar rats weighing 200–250 g and male Swiss albino mice weighing 25–40 g. All animals (groups of eight) were kept under controlled conditions during the experiments. The rats were fasted 18 h prior to their use.

2.4. Carrageenan and dextran-induced rat paw edema

Methanol extract (125 and 250 mg kg^{-1}), standard drug (indomethacin: 25 mg kg^{-1}) and the vehicle (saline solution 0.9% w/v NaCl) were administered by the oral route 1 h before receiving the injection of 0.1 ml of carrageenan (1% w/v) or dextran (1.5% w/v) into the subplantar area of the right hind paw. The volume (ml) of the paw was determined with a plethysmometer (LI-7500, Letica), as described by Winter et al. [10]. The volume was measured before the injection and at 1, 3, 5 h for carrageenan and 1, 2 and 3 h for dextran. The edema was reported as the difference between the final and the initial volume of the paw. The anti-inflammatory effect was expressed as the percentage inhibition caused by the extract in comparison with the vehicle-treated animal.

2.5. TPA-induced mouse ear edema [11]

An edema was induced on the right ear by topical application of 2.5 μg of TPA in 20 μl of acetone. The left ear (control) received the vehicle (acetone or 70% aq. EtOH). Methanol extract dissolved in 70% aq. EtOH were topically applied (0.5 mg ear^{-1}) simultaneously with TPA. The edema was calculated by the difference between the inflamed and not inflamed ear 4 h after the induction of inflammation. The anti-inflammatory activity was expressed as the percentage inhibition caused by the extract in comparison with the vehicle treated group. A reference group was treated with indomethacin (0.5 mg ear^{-1}).

2.6. Myeloperoxidase assay

Myeloperoxidase activity was determined on the supernatants from the homogenates of the ear biopsy [12]. The tissues placed in 1.5 ml of 50 mM sodium phosphate buffer, pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide (HTAB) were homogenized for 45 s at 0°C in a motor-driven homogenizer (Polytron PT 1200).

For the assay of myeloperoxidase, we have followed the method of Bradley et al. [13] modified for lecturing in a microplate reader. The following reagents were added in the order stated to wells of a 96-well microtiter plate: 50 μl of supernatant, 50 μl pH 6.0 phosphate buffer containing 0.5% HTAB, 50 μl *o*-dianisidine 0.68 mg ml^{-1} in distilled water, and to start the reaction 50 μl of freshly prepared 0.003% hydrogen peroxide. The optical density at 450 nm was read immediately and thereafter at 5-min intervals. The amount of enzyme in the samples was obtained by comparison of the rate of reaction with that in wells containing supernatant from the control group treated only with TPA.

2.7. Statistical evaluation

Values of edema reduction are expressed as mean ± SEM. The statistical significance of changes was analyzed using Student's *t*-test. $P < 0.05$ were considered to be significant.

Table 1
Anti-inflammatory effect of methanol extract from the leaves of *P. racemosa* var. *ozua* against rat paw carrageenan induced edema

Groups	Dose (mg kg^{-1})	Paw volume increase (ml)			Inhibition (%)		
		1 h	3 h	5 h	1 h	3 h	5 h
Control		0.63 ± 0.05	1.18 ± 0.08	1.29 ± 0.09			
Methanol extract	125	0.42 ± 0.02 **	0.68 ± 0.05 ***	0.59 ± 0.04 ***	33.3	42.4	54.3
	250	0.31 ± 0.04 ***	0.53 ± 0.05 ***	0.42 ± 0.04 ***	50.2	55.08	67.44
Indomethacin	25	0.16 ± 0.03 ***	0.15 ± 0.06 ***	0.20 ± 0.06 ***	74.6	87.3	84.5

** $P<0.01$.
*** $P<0.001$.

Table 2
Anti-inflammatory effect of methanol extract from the leaves of *P. racemosa* var. *ozua* against rat paw dextran induced edema

Groups	Dose (mg kg^{-1})	Paw volume increase (ml)			Inhibition (%)		
		1 h	2 h	3 h	1 h	2 h	3 h
Control		1.22 ± 0.08	1.17 ± 0.04	1.13 ± 0.09			
Methanol extract	125	1.58 ± 0.11	1.49 ± 0.09	1.30 ± 0.05	0.00	0.00	0.00
	250	1.04 ± 0.10 *	1.12 ± 0.09	1.20 ± 0.11	14.65	4.27	0.00
Indomethacin	25	1.21 ± 0.08	1.16 ± 0.08	1.11 ± 0.15	0.82	0.85	1.77

* $P<0.025$.

3. Results and discussion

Lupeol (4.8 mg) was isolated and identified, for the first time, from the methanol extract of the leaves of *Pimenta racemosa* var. *ozua* by chromatographic procedures, spectral and physical data as described in Section 2.

The antiedematous effects of the methanol extract on rat paw edema induced by carrageenan are shown in Table 1 and their effects on the inflammation provoked by dextran is presented in Table 2.

It is well established that carrageenan and dextran induce paw edema by different mechanisms. The initial phase of carrageenan edema is mediated by histamine and serotonin, while the mediators in the later phase are suspected to be arachidonate metabolites producing an edema dependent on mobilization of neutrophils. It seems that the primary effect of carrageenan as an inflammatory agent is the activation of phospholipase A_2 though its cytotoxic effect may initiate further inflammation [14]. Dextran is a polysaccharide of high molecular weight that induces an anaphylactoid reaction after injection in mice and rats extremities, characterized by extravasation and edema formation, due to mast degranulation with little protein and few neutrophils [15,16].

In relation to the extract assayed, in the carrageenan test, edematous response was significantly and dose-dependently suppressed in rats at all time intervals and with both doses. On the contrary the extract was not able to reduce the dextran-induced edema. This behavior was similar to the standard drug used, indomethacin, that did not exhibit significant activity against dextran induced edema, in accordance with the results obtained by other authors who demonstrated that cyclo-oxygenase (COX) inhibitors are unable to attenuate the dextran response [17,18].

Table 3 shows antiedematous activity induced by topical administration of the extract on ear edema provoked by local application of TPA. The majority of the activities of this phorbol ester appear to involve or depend on arachidonic release and metabolism, which may occur simultaneously with the interaction of TPA with a receptor site on protein kinase C (PKC). In this test, the extract significantly inhibited the inflammation ($P < 0.001$). Topical application of TPA induces a transient increase in prostanoid production. In this model, topically administered inhibitors of CO appear to be

Table 3
Anti-inflammatory topical effect of methanol extract from the leaves of *P. racemosa* var. *ozua* against TPA induced edema

Groups	Dose (mg ear)	Weight edema	Inhibition (%)
Control		15.88 ± 0.85	
Methanol extract	0.5	8.7 ± 0.47 ***	45.21
	1	7.03 ± 0.61 ***	55.73
	3	4.58 ± 0.87 ***	71.16
Indomethacin	0.5	1.98 ± 0.18 ***	87.53

*** $P<0.001$.

Table 4
MPO activity of the methanol extract from the leaves of *P. racemosa* var. *ozua* in the tissue inflammed by TPA

Groups	Dose (mg ear)	Absorbance increase	Enzyme inhibition (%)
Control		$9.10\times10^{-2}\pm2.12\times10^{-2}$	
Methanol extract	0.5	$1.96\times10^{-2}\pm5.20\times10^{-3}$ *	78.46
	1	$4.20\times10^{-3}\pm2.48\times10^{-3}$ **	95.38
	3	$6.20\times10^{-3}\pm4.07\times10^{-3}$ **	93.19
Indomethacin	0.5	$7.67\times10^{-3}\pm2.17\times10^{-3}$ **	91.57

* $P<0.025$.
** $P<0.01$.

more effective at inhibiting the edema response than LO inhibitors [19]. This result again induces the thought that the methanol extract has a behavior similar to COX inhibitors in this inflammatory model.

The MPO activity was strongly reduced by the extract and the compound (Table 4) and this indicates that they have strong effects on the cellular migration, mainly due to polymorphonuclear leukocytes.

Summarizing, our work suggests that the methanol extract from the leaves of *Pimenta racemosa* var. *ozua* possesses interesting anti-inflammatory activity against acute edema, both when orally administered and topically applied. It seems quite logical to think that the anti-inflammatory mechanism of *P. racemosa* is in part similar to the COX-selective inhibitor drugs, because its action is markedly influenced by the inhibition of neutrophil migration. These results also justify their use in folk medicine, although further pharmacological investigations are being carried out in order to understand the involvement of this triterpene in the anti-inflammatory action and to complete the study of this interesting species.

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