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Analgesic and Anti-inflammatory Effects of *Mangifera indica* L. Extract (Vimang)

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Vimang is an aqueous extract of *Mangifera indica* used in Cuba to improve the quality of life in patients suffering from elevated stress. To assess its possible analgesic and antiinflammatory effects, the results of a standard extract evaluation are presented. Analgesia was determined using acetic acid-induced abdominal constriction and formalin-induced licking. Antiinflammatory effects were evaluated using carrageenan- and formalin-induced oedema. Vimang (50–1000 mg/kg, p.o.) exhibited a potent and dose-dependent antinociceptive effect against acetic acid test in mice. The mean potency (DE_{50}) was 54.5 mg/kg and the maximal inhibition attained was 94.4%. Vimang (20–1000 mg/kg, p.o.) dose-dependently inhibited the second phase of formalin-induced pain but not the first phase. The DE_{50} of the second phase was 8.4 mg/kg and the maximal inhibition was 99.5%, being more potent than indomethacin at doses of 20 mg/kg. Vimang (20–1000 mg/kg, p.o.) significantly inhibited oedema formation ($p < 0.01$ or $p < 0.05$) of both carrageenan- and formalin-induced oedema in rat, guinea-pigs and mice (maximal inhibitions: 39.5, 45.0 and 48.6, respectively). The inhibitions were similar to those produced by indomethacin and sodium naproxen, p.o. The different polyphenols found in Vimang could account for the antinociceptive and antiinflammatory actions reported here for the first time for *M. indica* bark aqueous extract.

Keywords: Mangifera indica; analgesia; antiinflammatory agent; inflammation models.

INTRODUCTION

Mangifera indica L. (Anacardiaceae) grows in tropical and subtropical regions and its parts are commonly used in folk medicine for a wide variety of remedies (Coe and Anderson, 1996). The chemical composition of this plant has been studied extensively over the past years and the extracts yield triterpenes, flavonoids, phytosterols and polyphenols, in general (Anjaneyulu *et al.*, 1994; Khan *et al.*, 1994; Saleh and El Ansari,1975). These compounds have been reported as having cytotoxic, antineoplastic (Muanza *et al.*, 1995; Guha *et al.*, 1996), antioxidant (Born *et al.*, 1996), antiinflammatory and antibacterial activity (Das *et al.*,1989; Tona *et al.*, 1998). In Cuba, the aqueous extract of this species is used to improve the quality of life in patients suffering from elevated stress (Guevara *et al.*, 1998). The ethnomedical uses of this plant and the need to establish its pharmacological effects have promoted our present interest. The analgesic and antiinflammatory properties of a standard extract of *Mangifera indica* L. are supported from the results presented here.

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MATERIAL AND METHODS

Plant material. *Mangifera indica* L. was collected from a cultivated field located in the region of Pinar del Rio, Cuba. Voucher specimens of the plant were deposited in the Department of Technology, Center of Pharmaceutical Chemistry, La Habana, Cuba.

Preparation of Vimang extract. Stem bark extract of *Mangifera indica* L. was prepared by decoction with a polar solvent for 1 h. The extract was concentrated by evaporation and spray dried to obtain a fine brown powder (Vimang) which melts at 215–218 °C with decomposition. The chemical composition of this extract has been reported elsewhere (Center of Pharmaceutical Chemistry,1998).

Animals. Sprague Dawley rats and OF-1 mice were obtained from Centro para la Producción de Animales de Laboratorio (CENPALAB, La Habana, Cuba). Pirbright guinea-pigs and CF-1 mice were purchased from the Public Health Institute, Santiago, Chile. They were kept in a temperature controlled environment (23 °C) with a 12 h light–dark cycle, relative humidity 40–70%, with food and water *ad libitum* and fasted overnight (18 h) before the day of the experiments.

Abdominal constriction response caused by acetic acid. Male CF-1 mice (20–25 g) received Vimang

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Table 1. Effect of Vimang on acetic acid-induced abdominal constrictions in mice

Treatment	Dose (mg/kg)	Number of constrictions	Inhibition (%)
Vimang	0	42.5 ± 1.2	0
	50	20.9 ± 3.7[a]	50.8
	100	17.9 ± 3.3[a]	57.9
	200	8.5 ± 1.8[a]	80.0
	1000	2.4 ± 0.5[a]	94.4
ED_{50} (mg/kg)		54.5	—
(95% confidence limits)		(35.7 − 83.0)	
Sodium naproxen	12.5	12.8 ± 1.5[a]	70.0

Each group represents the mean ± SEM of 7–10 animals.
[a] $p < 0.05$ compared with control value. Values are expressed as percent inhibition of constriction index of treated animals with respect to control group.

(50–1000 mg/kg), water as a vehicle or naproxen (12.5 mg/kg) as a positive control 1 h prior to acetic acid injection. They were treated p.o. at a dose volume of 10 mL/kg. The abdominal constriction resulting from intraperitoneal injection of acetic acid (0.6%), consisting of a contraction of the abdominal muscle together with a stretching of hind limbs, was carried out according to procedures described previously (Davies *et al.*, 1997). After challenge, each animal was placed in separate glass cylinders and the number of abdominal constrictions was cumulatively counted over period of 30 min. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions between control animals and mice pre-treated with Vimang.

Formalin- induced licking and paw oedema. Male OF1 mice (25–30 g) were treated (dose volume 10 mL/kg, p.o.) with water, indomethacin (50 mg/kg) or Vimang (20–1000 mg/kg) 1 h before formalin injection. The procedure was similar to that described previously (Santos *et al.*, 1994). Briefly, 20 μL 2.5% formalin (0.92% formaldehyde) made up in saline solution was injected under the surface of the right hind paw. Two mice (control and treated) were observed simultaneously from 0 to 30 min after formalin injection. The initial nociceptive scores normally peaked 5 min (first phase) and 15–30 min after formalin injection (second phase), representing the tonic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987). After intraplantar injection of formalin, the animals were immediately placed into a glass cylinder 20 cm in diameter, and the amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. At the end of all experiments the animals were killed by cervical dislocation and the paws were cut at the knee and weighed (mg) on an analytical balance. Oedema was determined by the increase of weight of the right hind paw versus the left hind paw.

Carrageenan-induced paw oedema. This test was carried out as described by Boughton-Smith *et al.* (1993). Male and female Sprague Dawley rats (250–300 g) received water, Vimang (20–1000 mg/kg) or indomethacin (10 mg/kg) as a positive control. They were treated p.o. at a dose volume of 5 mL/kg, 1 h before subplantar injection in the right hind paw of carrageenan (0.1 mL of 1% suspension in 0.9% saline). Five hours after injection the animals were killed by ether anaesthesia, the paws were rapidly amputated at the knee and weighed (mg) on an analytical balance. Oedema was determined by the increase of weight of the right hind paw versus the left hind paw.

Pirbright guinea-pigs (200–300 g) received Vimang (1000 mg/kg) or naproxen (4 mg/kg) as a positive control. They were treated p.o. at a dose volume of 4 mL/kg, 1 h before subplantar injection in the right hind paw of carrageenan (0.1 mL of 1% suspension in 0.9% saline). Paw thickness was measured with an Ugo Basile plethysmometer (model 7150) immediately and 3 h after injection of carrageenan, according to the method of Delporte *et al.* (1998). Oedema was expressed as the increase in paw thickness (in vol.) measured after carrageenan injection and compared with the pre-injection value for individual animals.

Statistical analysis. The results are presented as mean ± SEM and the statistical significance between the groups was determined by means of analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, except for carrageenan-induced paw oedema in the guinea-pig data, which were compared using the Wilcoxon test for unpaired data. p values less than 0.05 ($p < 0.05$) were considered as indicative of significance. When appropriate, the DE_{50} was calculated using GraphPad InPlot software (GraphPad Sofware Inc., version 4.03, 1992).

RESULTS

Abdominal constriction response caused by acetic acid

To investigate the analgesic activity of *M. indica*, a standard aqueous extract (Vimang) prepared from the bark of this plant was investigated for inhibitory effect on acetic acid-induced abdominal constriction. As can be seen in Table 1, this extract exhibited a potent and dose-dependent antinociceptive effect against acetic acid-induced writhing response in mice. The calculated DE_{50} value was 54.5 mg/kg. At 1000 mg/kg, Vimang almost completely abolished the irritant pain of the test (94.4% of inhibition). Sodium naproxen (12.5 mg/kg) also exhibited a potent antinociceptive effect.

Formalin-induced licking

Vimang dose-dependently inhibited the second phase but not the first phase of formalin-induced pain (Table 2). The maximal inhibition for the second phase was 99.5% and the DE_{50} was 8.4 mg/kg. In this case, indomethacin (50 mg/kg) also inhibited the second phase of formalin-induced pain ($p < 0.01$).

Carrageenan- and formalin-induced paw oedema

Vimang significantly inhibited oedema formation ($p < 0.01$ or $p < 0.05$) of both carrageenan- and formalin-induced oedema (Table 3). These inhibitions were similar to that produced by the non-steroidal antiinflammatory drugs used as a positive control (indomethacin and sodium naproxen, p.o.). The maximal inhibitions

Table 2. Effect of Vimang against the first phase (0–5 min) and the second phase (15–30 min) in the formalin test of mice

Treatment	Dose (mg/kg)	Licking (s) 0–5 min	Licking (s) 15–30 min
Vimang	0	154.3 ± 9.5	144.7 ± 8.2
	20	139.7 ± 7.7	58.7 ± 9.9*
	50	133.2 ± 8.7	10.2 ± 3.5*,a,b
	100	130.8 ± 5.0	8.5 ± 4.6*,a,b
	200	128.9 ± 8.6	7.9 ± 2.8*,a,b
	1000	131.0 ± 8.2	0.7 ± 0.5*,a,b
ED_{50} (mg/kg)		—	8.4
(95% confidence limits)			(1.3–55.0)
Maximal inhibition (%)		16.5	99.5
Indomethacin	50	176.3 ± 9.0	52.5 ± 6.9*

Each group represents the mean ± SEM of 6–8 animals.
*,a,b $p < 0.01$ compared with control, indomethacin and Vimang (20 mg/kg) values, respectively.

were 39.5%, 45.0% (rats and guinea-pigs, respectively in carrageenan-induced oedema) and 48.6% (mice in formalin-induced oedema).

DISCUSSION

The association of antioxidants and inflammation stems from the recognition that free radicals are produced during the inflammatory process by macrophages, it is reported that oxygen reactive species are involved in the cyclooxygenase- and lipooxygenase-mediated conversion of arachidonic acid into proinflammatory intermediates (Backhouse *et al.*, 1994). On this basis, several natural and synthetic antioxidants (Swingle *et al.*, 1985; Calixto *et al.*, 1998) have been tested and shown to possess analgesic and antiinflammatory properties.

Chemical studies performed with Vimang have enabled the isolation and identification of phytosterols and polyphenolic compounds (Center of Pharmaceutical Chemistry,1998). The antiinflammatory and analgesic activity of these compounds are of common occurrence in many plants (Delporte *et al.*, 1998; Calixto *et al.*, 1998; Cechinel-Filho *et al.*, 1996). An active antiinflammatory effect of *M. indica* seed kernel in acute, proliferative and immunological inflammation has been elsewhere reported (Das *et al.*,1989).

It has also been reported that the active metabolite (norathyriol) of the major phenolic component of Vimang (mangiferin) inhibited concentration-dependently the formylmethionyl-leucyl-phenylalanine (fMLP)-induced superoxide anion generation and oxygen consumption in rat neutrophils (Hsu *et al.*, 1997).

On the other hand, recent studies have shown that formalin releases several mediators of inflammation (Hunskaar *et al.*, 1986; Hunskaar and Hole, 1987).

The formalin-induced pain test defines two distinct periods of response, i.e. 'early response' or first phase and 'late response' or second phase (Hunskaar *et al.*, 1986a). Detailed discussion of the 'late phase' has been limited to the mechanism of action, which appears attributable to inhibition of prostaglandin synthesis on pain modulation. However, the second phase (inflammatory pain response), but not the first phase (tonic pain response) of the formalin test can be attenuated in a dose-dependent fashion by drugs, such as aspirin, paracetamol or indomethacin, known to inhibit cyclooxygenase activity (Hunskaar and Hole, 1987; Calixto *et al.*, 1998). Vimang, an aqueous extract of *M. indica* bark, exhibits a potent action in the second phase of this test, being more potent than indomethacin from doses greater than 20 mg/kg (Table 2). These results strongly support the view that the

Table 3. Effect of Vimang on oedema paw in the formalin and carrageenan tests

Treatment	Dose (mg/kg)	Oedema inhibition (%) Formalin (Mice)	Carrageenan (Rats)	Carrageenan (Guinea-pigs)
Vimang	20	27.3[b]	23.7[b]	na
	50	35.3[a]	24.5[b]	na
	100	36.6[a]	27.8[a]	na
	200	48.4[a]	33.5[a]	na
	1000	48.6[a]	39.5[a]	45.0[b]
Indomethacin	10	na	61.1[a]	na
	50	43.9[a]	na	na
Sodium naproxen	4	na	na	54.6[a]

Values are expressed as percent inhibition of oedema index of treated animals with respect to control groups. Each group represents the mean ± SEM of 6–11 animals.
[a] $p < 0.01$;
[b] $p < 0.05$ compared with respective control values. na, not assayed.

active principles present in Vimang are much more effective in attenuating hyperalgesic or persistent pain, than the neurogenic pain caused by formalin.

Moreover, we have used the carrageenan-induced paw oedema as an *in vivo* model of inflammation (Winter *et al.*, 1963) because it is a screening procedure in which the involvement of the cyclooxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established (Smith *et al.*, 1974). It is reported that the carrageenan oedema shows three distinct phases, namely an initial release of histamine and 5-hydroxytryptamine, a second phase mediated by kinins and a third phase (about 5 h of oedema) in which the mediator is suspected to be prostaglandin (Wang and Mineshita, 1996). Vimang, dependently of dose, inhibited the carrageenan paw oedema in a similar manner to indomethacin in rats at 5 h. Meanwhile, at 3 h the effect of Vimang on hind paw oedema in the guinea-pigs was comparable to naproxen. Vimang also inhibited formalin-induced hind paw oedema compared with indomethacin in mice. Thus, the fact that Vimang inhibited the inflammatory oedema induced by irritant agents may be interpreted by the extract modulating the B_2 receptors against bradykinin, prostaglandin receptors or the liberation of these substances (Corrêa and Calixto, 1993; Décarie *et al.*,1996).

Taken together, our current findings support the view that different classes of constituents reported for Vimang, such as steroids, flavonoids and tannins could account for the antinociceptive and antiinflammatory actions by means of inhibiting the synthesis of prostaglandin or products of arachidonic acid metabolism and the production of reactive oxygen species.

Further research is being accomplished in our laboratories to elucidate their mechanisms of action and to characterize the active principles contained in Vimang.

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