In vivo and in vitro anti-inflammatory activity of *Mangifera indica* L. extract (VIMANG®)

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Abstract

A standard aqueous extract of *Mangifera indica* L., used in Cuba as an antioxidant under the brand name of VIMANG®, was tested in vivo for its anti-inflammatory activity using commonly accepted assays. *M. indica* extract, administered topically (0.5–2 mg per ear), reduced ear edema induced by arachidonic acid (AA) and phorbol myristate acetate (PMA, $ED_{50} = 1.1$ mg per ear) in mice. In the PMA model, *M. indica* extract also reduced myeloperoxidase (MPO) activity. This extract p.o. administered also inhibited tumor necrosis factor alpha (TNFα) serum levels in both models of inflammation (AA, $ED_{50} = 106.1\ mg\ kg^{-1}$ and PMA, $ED_{50} = 58.2\ mg\ kg^{-1}$). In vitro studies were performed using the macrophage cell line RAW264.7 stimulated with pro-inflammatory stimuli (LPS–IFNγ or the calcium ionophore A23187) to determine PGE_2 or LTB_4 release, respectively. The extract inhibited the induction of PGE_2 with $IC_{50} = 64.1\ \mu g\ ml^{-1}$ and LTB_4 $IC_{50} = 22.9\ \mu g\ ml^{-1}$. *M. indica* extract also inhibited human synovial secretory phospholipase (PL)A_2 with $IC_{50} = 0.7\ \mu g\ ml^{-1}$. These results represent an important contribution to the elucidation of the mechanism involved in the anti-inflammatory and anti-nociceptive effects reported by the standard *M. indica* extract VIMANG®.

Keywords: *Mangifera indica*; Mangiferin; Eicosanoids; PLA_2; Ear edema

1. Introduction

Mangifera indica L. (*Anacardiaceae*) grows in the tropical and subtropical region and its parts are commonly used in folk medicine for a wide variety of remedies [1]. Chemical studies performed with a standard aqueous extract of the stem bark from *M. indica*, which has been used in pharmaceutical formulations in Cuba under the brand name VIMANG®, have enabled the isolation and identification of phenolic acids (gallic acid, 3,4 dihydroxy benzoic acid, benzoic acid), phenolic ester (gallic acid methyl ester, gallic acid propyl ester, benzoic acid propyl ester), flavan-3-ols (catechin and epicatechin) and the xanthone mangiferin, which is the predominant component of this extract (10%). VIMANG® is also rich in fatty acid as myristic, palmitic, stearic, oleic–linoleic and eicosatrienoic [2]. The anti-inflammatory activity of many of these compounds is widely known, while they are of common occurrence in several plants [3]. On the other hand, it has been suggested that many anti-inflammatory drugs may exert some of their effects by scavenging oxidants, and decreasing formation of reactive oxygen species (ROS) by activated phagocytes [4]. Recently, the first analgesic and anti-inflammatory effects of VIMANG® were reported. This extract inhibited the abdominal constriction induced by acetic acid and formalin-induced licking in mice, and the edema induced by carrageenan and formalin in mice, rats and guinea-pigs [5]. Additionally, it has been demonstrated that *M. indica* extract has a powerful scavenger activity against hydroxyl radicals and hypochlorous acid. This extract also presented a significant inhibitory effect on the peroxidation of rat-brain phospholipid and inhibited DNA damage by bleomycin or copper phenanthroline systems [6]. A comparative study was performed concerning the protective abilities of the extract, mangiferin and others well known antioxidants against the tetradecanoylphorbol acetate-induced oxidative damage in serum, liver, brain as well as in the over-production of reactive oxygen species

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by peritoneal macrophages and it could be concluded that the stem bark extract of *M. indica* prevented the production of ROS and the oxidative tissue damage in vivo while it was more active than vitamin C, vitamin E, mangiferin and β-carotene [7].

Taking into account the previous findings, it was decided to test the potential anti-inflammatory activity of a standard stem bark *M. indica* aqueous extract (VIMANG®) in several in vivo and in vitro experimental models, in order to characterize the role of this extract in affecting the inflammatory process.

2. Materials and methods

2.1. Drugs

M. indica was collected from a cultivated field located in the region of Pinar del Rio, Cuba. Voucher specimens of the plant (code: 41722) were deposited at the Herbarium of Academy of Sciences, guarded by the Institute of Ecology and Systematic from Ministry of Science, Technology, and Environmental, La Habana, Cuba. Stem bark extract of *M. indica* L. was prepared by decoction for 1 h. The extract was concentrated by evaporation and spray dried to obtain a fine brown powder, which was used as the standardized active ingredient of VIMANG® formulations. It melts at 210–215 °C with decomposition. The chemical composition of this extract has been characterized by chromatographic (planar, liquid and gas) methods, mass spectrometry and UV-Vis spectrophotometry [2].

2.2. Reagents

Arachidonic acid (AA), phorbol myristate acetate (PMA), indomethacin, dexamethasone, dimethyl sulfoxide (DMSO), interferon gamma (IFNγ), bacterial lipopolysaccharide (LPS), calcium ionophore A23187, *O*-dianisidine, nordihydroguaiaretic acid (NDGA), and all other reagents were obtained from Sigma (St. Louis, MO, USA). Human synovial recombinant PLA_2 enzyme and Petrosaspongiolide M (P-M) were kindly provided by Dr. M. Payá (University of Valencia, Spain). P-M was used as inhibitor of PLA_2 [8]. Mangiferin (2-β-D-glucopyranosyl-1,3,6,7-tetra-hydroxy-9H-xanthen-9-one) was supplied by the Laboratory of Analytical Chemistry, Center of Pharmaceutical Chemistry (Cuba). It was purified from *M. indica* stem bark standardized extract by extraction with methanol and its purity (90%) was assessed [2].

2.3. Animals

Male Swiss mice weighing 25–30 g from Centro para la Producción de Animales de Laboratorio (CENPALAB, La Habana, Cuba) and Instituto de Salud Pública (Santiago, Chile) were used in these studies. They were kept in a temperature controlled environment (23 °C) with a 12-h light:12-h dark cycle, relative humidity 40–70%, with food and water ad libitum and fasted overnight (18 h) before the day of the experiments. The experiments were conducted in accordance with the ethical guidelines for investigations with laboratory animals and were approved by the Ethical Committee for Animal Experimentation of the Center of Pharmaceutical Chemistry.

2.4. AA-induced mouse ear edema

The method described by Romay et al. [9] with some modifications was followed. Inflammation was induced by topical application of AA (2 mg in 20 μl of acetone) of both surfaces of the right ear of each mouse. Left ear (control) received the vehicle. *M. indica* extract was administered topically (0.5, 1 and 2 mg per ear in DMSO) 1 h before AA. Two control groups were used: a group with application of AA on the right ear and a positive control group that received nimesulide (1 mg per ear in 20 μl acetone). Inflammation was followed for 1 h and thereafter animals were killed by cervical dislocation. A 6 mm section from each ear was removed with a metal punch and weighed. Ear edema was calculated by subtracting the weight of the left ear (vehicle) from the right ear (treatment), and was expressed as edema weight. Inhibition percentage was expressed as a reduction in weight with respect to the control group.

2.5. PMA-induced mouse ear edema

According to a modified method of Griswold et al. [10], 4 μg per ear of PMA, in 20 μl of acetone, was applied to both surfaces of the right ear of each mouse. The left ear (control) received the vehicle (acetone and/or DMSO, 20 μl). *M. indica* extract was administered topically (0.5, 1 and 2 mg per ear in DMSO) 1 h before PMA application. Two control groups were used: a control group with the application of PMA on the right ear and the reference group was treated with indomethacin (1 mg per ear in 20 μl acetone). Six hours after PMA application, mice were killed by cervical dislocation and a 6 mm diameter disc from each ear was removed with a metal punch and weighed. Ear edema was calculated by subtracting the weight of the left ear (vehicle) from the right ear (treatment), and was expressed as edema weight. Inhibition percentage was expressed as a reduction in weight with respect to the control group.

2.6. MPO assay

Tissue samples of each ear, from the PMA model, were assessed biochemically with the neutrophil marker enzyme, myeloperoxidase (MPO), using the method of Bradley et al. [11] with minor modifications. All the ear tissue was homogenized in 50 mM K_2HPO_4 buffer (pH 6) containing 0.5% hexadecyl trimethylammonium bromide

(HTBA) using a Polytron (Ultra-turrax T-25) homogenizer. After freeze-thawing for three times, the samples were centrifuged at 2500 × *g* for 30 min at 4 °C and the resulting supernatant assayed spectrophotometrically for MPO determination. In brief, 40 μl of sample was mixed with 960 μl of 50 mM phosphate buffer pH 6, containing 0.167 mg ml^{-1} *O*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured with a Spekol 220 spectrophotometer (Carl Zeiss, Germany). MPO activity data are presented as units per mg of tissue. One unit of MPO activity was defined as that degrading 1 μmol of peroxide per minute at 25 °C.

2.7. TNFα assay

An hour after administration of *M. indica* extract (50, 100 and 200 mg kg^{-1} by gavage) AA (2 mg per ear) or PMA (4 μg per ear) was applied to both surfaces of the right ear of mice. Left ears received the vehicle (acetone). Dexamethasone (3 mg kg^{-1}, i.p.), used as a reference drug, was administered in 0.2 ml saline 30 min before AA or PMA. After 1 (AA) or 6 h (PMA) of application the animals were anesthetized with ether atmosphere and the blood was extracted from retro-orbital cavity and the serum obtained for TNF determination.

Serum tumor necrosis factor alpha (TNFα) was measured with a cytotoxicity bioassay, using L929 cell line in the presence of 1 μg ml^{-1} of actinomycin D [12]. Recombinant TNFα was used as standard (BASF/Knoll, Ludwigshafen, Germany; specific activity 10^7 U mg^{-1}) and expressed as pg ml^{-1}.

2.8. Cell viability

Preliminary cytotoxicity tests were performed in order to assess if the influence of drugs on the eicosanoid release should be due to a cytotoxic effect. Cell viability was assessed by using a MTT-based colorimetric assay [13]. 8 × 10^5 cells diluted with Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal calf serum (FCS) were pipetted into 96-well microtiter plates, and were incubated overnight at 37 °C and 5% CO_2. The cells were exposed to various concentrations of samples for 3 h under the same conditions of incubation. MTT solution dissolved in phosphate buffered saline was added to the wells at 1 mg ml^{-1} final concentration. After carefully aspirating the medium 100 μl of DMSO were added for the dissolution of formazan crystals. The absorbance of each well was then read at 520 nm using a microplate reader. The values of the maximum non-toxic concentration obtained were used in PGE_2 and LTB_4 determination.

2.9. Assay of PGE_2 and LTB_4 release in activated macrophages

RAW264.7 cell line was suspended in DMEM supplemented with 10% FCS, and seeded into 24-well plates at a concentration of 5 × 10^5 cells ml^{-1}. After adhering to plates (24 h at 37 °C in an atmosphere of 5% CO_2), non-adherent cells were washed off, and the cells (90% of adherent cells) received fresh DMEM (without FCS). Cells were pre-treated for 1 h at 37 °C with the test compounds or vehicle, and then stimulated further for 4 h by adding calcium ionophore A23187 (final concentration 10^{-6} M, for LTB_4 release) or 24 h with LPS–IFNγ (100 ng ml^{-1} and 10 U ml^{-1}, for PGE_2 release) respectively, according with Hulkower et al. [14]. Controls contained only DMSO (basic level of released eicosanoid) or reference compound (total inhibition of eicosanoid release). The contents of all dishes were frozen at −20 °C and retained for analysis for PGE_2 and LTB_4 by means of a radioimmunoassay according to the procedure described by the manufacturer (Amersham).

2.10. Assay of secretory PLA_2

Secretory PLA_2 was assayed by using [^{3}H]oleate-labeled autoclaved *Escherichia coli* following a modification of the method of Escrig et al. [15]. *E. coli* strain CECT 101 were seeded in medium containing 1% tryptone, 0.5% NaCl and 0.6% sodium dihydrogen orthophosphate, pH 5.0, and grown for 6–8 h at 37 °C with vigorous oxygenation in the presence of 5 μCi ml^{-1} [^{3}H]oleic acid (sp. act. 10 Ci mmol^{-1}). Then, the bacteria were pelleted by centrifugation at 2550 × *g* for 10 min, and the cells were extensively washed in buffer (0.7 M Tris–HCl, 10 mM $CaCl_2$, 0.1% bovine serum albumin, pH 8.0) to remove unincorporated or loosely-bound oleate. The washed *E. coli* membranes were resuspended in saline and autoclaved for 30–45 min under full pressure, washed and centrifuged again and stored at −20 °C until needed. At least 95% of the radioactivity was incorporated into phospholipids.

For the assay of PLA_2, human recombinant synovial enzyme was diluted in 10 μl of 100 mM Tris–HCl, 1 mM $CaCl_2$, pH 7.5. The enzyme was pre-incubated at 37 °C for 5 min with 2.5 μl of *M. indica* extract at the concentration assayed (0.01–100 μg ml^{-1}), mangiferin (10 μg ml^{-1}), Petrosaspongiolide M (5 μM), or its vehicle in a final volume of 250 μl. Incubation proceeded for 15 min at 37 °C in the presence of 10 μl of autoclaved oleate-labeled membranes and was terminated by addition of 100 μl ice-cold solution of 0.25% bovine serum albumin in saline to a final concentration of 0.07% (w/v), to bind the released [^{3}H]-oleic acid. The assay mixture was then centrifuged at 2500 × *g* for 10 min at 4 °C, and the radioactivity in the supernatant was determined by liquid scintillation counting.

2.11. Statistical analysis

PGE_2 and LTB_4 concentrations were assessed directly from a standard curve. The results are presented as the mean ± S.E.M. and statistical significance between the groups was determined by means of one-way analysis of

variance (ANOVA) followed by unpaired Student's *t*-test to determine statistical significance. *P* values less than 0.05 ($P < 0.05$) were considered as indicative of significance. Regression analysis was used to calculate the effective dose 50 (ED_{50}), defined as the dosage of each drug necessary to produce a 50% inhibition on AA- and PMA-induced ear edema. The inhibitory concentration 50 (IC_{50}) was calculated in the same manner.

3. Results

3.1. Edema induced by AA in mouse ear

M. indica extract significantly ($P < 0.05$) attenuated the AA-induced edema at all tested doses (0.5–2 mg per ear) 1 h after AA application (Table 1). Nimesulide also inhibited the edema.

3.2. Edema induced by PMA in mouse ear and MPO activity

It can be seen in Table 1 that PMA-induced inflammation was also inhibited by *M. indica* extract when it was administered topically ($ED_{50} = 1.1$ mg per ear), 1 h before PMA application. The extract also inhibited significantly ($P < 0.05$) MPO activity induced by PMA (Table 2). Indomethacin also inhibited the inflammatory and MPO responses in this model.

3.3. TNFα serum levels

Both, AA and PMA, stimulated the TNFα production in the serum of mice. *M. indica* extract, administered p.o., inhibited TNFα serum dose-dependently in both models: in the AA model $ED_{50} = 106.1\ mg\ kg^{-1}$ (Fig. 1A) and in the PMA model $ED_{50} = 58.2\ mg\ kg^{-1}$ (Fig. 1B). Dexamethasone ($3\ mg\ kg^{-1}$, i.p.) also inhibited TNFα in both models of inflammation.

Table 2
Effect of *M. indica* extract on MPO induction in the mouse ear edema induced by PMA.

Treatment	Dose (mg per ear)	MPO (U mg per tissue)	Inhibition (%)
PMA	0.004	2.6 ± 0.6	–
M. indica	0.5	2.1 ± 0.4	19.2
	1	1.6 ± 0.1*	38.5
	2	1.5 ± 0.1*	42.3
Indomethacin	1	0.7 ± 0.1*	73.1

Right ear of mice has been stimulated with PMA to induce the respiratory burst and MPO release. *M. indica* extract and indomethacin were administered topically 1 h before PMA. Inflammation was followed for 6 h after application of PMA and thereafter animals were killed by cervical dislocation. Biopsies were extracted from both ears and MPO was determined using the method of Bradley et al. [11], according with the description in Section 2. Each group represents the mean ± S.E.M. of 7–10 animals.

* $P < 0.05$ statistical significance compared with PMA group (unpaired Student's *t*-test).

3.4. Effects of PGE_2 and LTB_4 release

M. indica extract, mangiferin, indomethacin and NDGA were not cytotoxic at any of the concentrations tested ($1–100\ \mu g\ ml^{-1}$).

As showed in Table 3, addition of LPS–IFNγ and calcium ionophore A23187 to RAW264.7 macrophages causes the generation of nanogram amounts of eicosanoids via both cyclooxygenase (COX) and lypoxygenase pathways. This effect was measured in terms of immunoassayable PGE_2 and LTB_4, respectively. Validation of this system for the identification of inhibitors of the two divergent pathways of arachidonate metabolism was performed by using indomethacin, a well characterized cyclooxygenase inhibitor (92.3% inhibition of PGE_2 at 100 μM) and NDGA, a known inhibitor of 5-lypoxygenase (97.4% inhibition of LTB_4 at 25 μM).

The compounds tested showed a considerable activity as inhibitors of eicosanoid release from LPS–IFNγ and calcium ionophore A23187 to RAW264.7 macrophages. The IC_{50}s for *M. indica* were $64.1\ \mu g\ ml^{-1}$ (PGE_2-release assay) and $22.9\ \mu g\ ml^{-1}$ (LTB_4-release assay). Mangiferin, about

Table 1
Effect of *M. indica* extract on edema induced by AA and PMA in mouse ear

Treatment (mg per ear)	Dose (mg per ear)	AA-induced ear edema		PMA-induced ear edema	
		Edema weight (mg)	Inhibition (%)	Edema weight (mg)	Inhibition (%)
AA	2	10.3 ± 0.5	–	–	–
PMA	0.004	–	–	10.2 ± 1.2	–
M. indica	0.5	6.0 ± 1.3*	41.6	6.6 ± 1.1*	35.7
	1	5.5 ± 1.7*	46.6	5.0 ± 0.7*	51.0
	2	5.2 ± 1.2*	49.7	5.7 ± 1.4*	44.7
Indomethacin	1	–	–	2.3 ± 0.7*	78.0
Nimesulide	1	5.3 ± 0.8*	49.0	–	–

M. indica extract, indomethacin, nimesulide and vehicles (acetone and/or DMSO) were administered topically 1 h before of AA or PMA on both surfaces of right ears of mice, according to Section 2. Inflammation was followed after application of AA or PMA, respectively, and thereafter animals were killed by cervical dislocation. Ear edema was calculated by subtracting the weight of biopsies of the left ear (vehicle) from right ear (treatment), and was expressed as edema weight. Each group represents the mean ± S.E.M. of 7–10 animals.

* $P < 0.05$ statistical significance compared with AA or PMA groups (unpaired Student's *t*-test).

Table 3
Effect of *M. indica* extract and mangiferin on PGE_2 and LTB_4 biosynthesis by RAW264.7 macrophages stimulated by LPS + IFNγ and A23187

	Concentration	PGE_2 (ng ml^{-1})	Inhibition (%)	LTB_4 (ng ml^{-1})	Inhibition (%)
LPS + IFNγ	100 ng ml^{-1} + 10 U ml^{-1}	182.0 ± 10.3	0	–	–
A23187	10^{-6} M	–	–	40.0 ± 6.3	0
M. indica	10 μg ml^{-1}	175.0 ± 0.3	3.8	23.6 ± 5.3*	41.0
	50 μg ml^{-1}	119.5 ± 8.6*	34.3	12.7 ± 2.6*	68.3
	100 μg ml^{-1}	30.6 ± 2.6*	83.2	5.0 ± 2.6*	87.5
Mangiferin	10 μg ml^{-1}	28.6 ± 3.5*	84.3	12.5 ± 3.5*	68.8
Indomethacin	100 μM	14.1 ± 3.2*	92.3	–	–
NDGA	25 μM	–	–	1.1 ± 0.4*	97.4

RAW264.7 cells were pre-treated for 1 h at 37 °C with *M. indica*, mangiferin, indomethacin, NDGA and vehicle (DMSO). Then, they were stimulated for a further 4 h by calcium ionophore A23187 (10^{-6} M) for LTB_4 release or 24 h by LPS (100 ng ml^{-1})–IFNγ (10 U ml^{-1}) for PGE_2 release, according to the procedure described in Section 2. LTB_4 and PGE_2 were determined by radioimmunoassay. All data values represent mean ± S.E.M. ($n = 6$).

* $P < 0.05$ compared with control groups (unpaired Student's *t*-test).

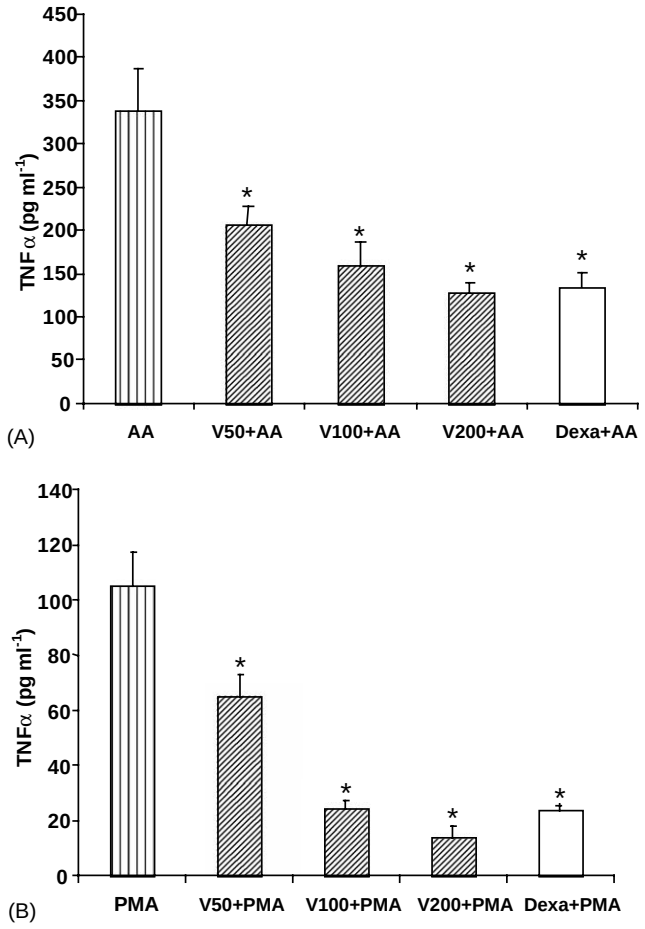

Fig. 1. Effect of *M. indica* extract on inhibition of systemic TNFα in mice with ear edema induced by AA or PMA. Mice were treated topically with AA (2 mg per ear) or PMA (4 μg per ear) according to the procedure described in Section 2. *M. indica* extract and vehicle (water) were administered orally 1 h before AA or PMA application. TNFα serum level was analyzed 1 or 6 h after AA (A), or PMA (B) application, respectively. Values are expressed as mean ± S.E.M. of 4–5 animals. * $P < 0.05$ statistical significance compared with control group (unpaired Student's *t*-test). V: *M. indica* (50, 100 and 200 mg kg^{-1}); Dexa: dexamethasone (3 mg kg^{-1}, i.p.) was administered 30 min before AA or PMA application.

Table 4
Inhibitory effects of *M. indica* extract and mangiferin on secretory PLA_2 activity

	Concentration	PLA_2 ($\times 10^3$ cpm per mg protein)	Inhibition (%)
Control	–	5.3 ± 0.4	0
M. indica	0.01 μg ml^{-1}	4.8 ± 0.2	14.5
	0.05 μg ml^{-1}	4.3 ± 0.2*	23.2
	0.1 μg ml^{-1}	4.2 ± 0.2*	27.2
	0.5 μg ml^{-1}	3.7 ± 0.1*	40.8
	0.75 μg ml^{-1}	3.1 ± 0.2*	52.1
	1.0 μg ml^{-1}	2.3 ± 0.1*	76.1
	10 μg ml^{-1}	1.5 ± 0.1*	96.5
	100 μg ml^{-1}	1.4 ± 0.1*	97.6
Mangiferin	10.0 μg ml^{-1}	1.6 ± 0.1*	92.8
P-M	5 μM	1.4 ± 0.1*	97.8

Enzyme was pre-incubated with *M. indica*, Petrosaspongiolide M (P-M) and vehicle (control) for 5 min at 37 °C, and after addition of substrate, incubation proceeded for 15 min. All data values represent mean ± S.E.M. ($n = 6$).

* $P < 0.05$ compared with control group (enzyme with vehicle), unpaired Student's *t*-test.

10% present in the extract, also exhibited a potent inhibitory effect.

3.5. Secretory PLA_2

As showed in Table 4, *M. indica* extract exerted inhibitory effects, dependent of concentration, on human recombinant synovial PLA_2 with $IC_{50} = 0.7$ μg ml^{-1}. Mangiferin and Petrosaspongiolide M (5 μM) also exhibited potent inhibitions of this enzyme.

4. Discussion

VIMANG® is an extract obtained from the stem bark of selected varieties of *M. indica* and contains a defined mixture of components: polyphenols, terpenoids, steroids, fatty acids and microelements [2]. Mangiferin (the main polyphenol of VIMANG®, 10%) has been tested in vitro for its antioxidant [16–18], immuno-stimulating and anti-viral

properties [19]. The mangiferin aglycone (norathyriol) was also tested in vitro as an inhibitor of the formylmethionyl-leucyl-phenylalanine induced respiratory burst in rat neutrophils [20]. Norathyriol has also been tested on A23187-induced pleurisy and analgesia in mice [21] and it was demonstrated that this aglycone inhibits AA metabolites like PGs and LTs in these models. The results suggested that norathyriol inhibited both cyclooxygenase and 5-lypoxigenase (LOX) pathway, and that this action probably accounts for its anti-inflammatory and analgesic effects.

Metabolism of AA by COX leads to the generation of PGs and thromboxanes that mediate pain and edema associated with inflammation [22]. The LOX pathway utilizes AA to produce LTs, including the leukocyte chemoattractant LTB_4. Owing to the contribution of LTs to the pathogenesis of many inflammatory processes, they also represent an important target for therapeutic regulation [23].

The in vivo model of AA mouse ear inflammation is very suitable and sensitive, although not specific, to test inhibitors of LOX. The direct topical application of AA results in the rapid onset of edema formation most probably related to LTC_4 [24] and LTB_4 synthesis [25]. In contrast, topical application of phorbol esters (like PMA) induce a long lasting inflammatory response associated with transient increase in prostanoid production and marked cellular influx. This high prostaglandin level is very likely due to COX induction [26].

The results of this study were consistent with these findings, and the *M. indica* extract showed an inhibitory effect on edemas induced by AA or PMA when these agents were administered topically. The extract could also exert this dual action because of its powerful antioxidant activity [7], since it has been shown that the treatment of mouse skin with protein kinase C (PKC) promoters, such as PMA, induces formation of free radicals in vivo. It has been also demonstrated that pre-treatment of mouse skin by antagonists of PKC may suppress inflammation and ROS formation [27]. ROS are also relevant for the synthesis of different mediators and thus it is known that these species may regulate the production of TNFα in some inflammatory responses [28]. The TNF released by mast cells can stimulate PLA_2 activity, which releases AA from phospholipids and stimulates the activity or the level of COX and LOX [29]. In the present study, oral administration of *M. indica* extract inhibited significantly the circulating TNFα produced when AA or PMA were administered on the ear of mice. This effect also shows the anti-inflammatory action of the extract.

In the PMA model, *M. indica* extract also reduced MPO activity, thus preventing the generation of oxidants such as hypochlorous acid. MPO is an enzyme present in neutrophils and at a much lower concentration in monocytes and macrophages. It is well known that the level of MPO activity is directly proportional to the neutrophil concentration on the inflamed tissue [11], by which measurement of the enzyme activity has been considered a quantitative and sensitive marker of chemotaxis and neutrophil infiltration in the inflammatory process [30]. As a result, the extract could decrease tissue damage caused by hydrolytic enzymes and besides by some oxidant species.

Perhaps, the anti-inflammatory effect in the AA and PMA models are not depending of the doses due to the low bioavailability of the extract components by the topical pathway in an acute treatment.

The inhibitory effects of *M. indica* are presented in this study on in vitro eicosanoid-releasing systems to investigate whether the extract contributes to this mechanism of anti-inflammatory activity. Many different studies have suggested that macrophages are a potent source of AA metabolites generated via COX and LOX pathways [27]. Moreover, macrophages participate in host defense, immunity and inflammatory responses, where they are potently activated resulting in the production of cytokines, oxygen and nitrogen species and eicosanoids. In macrophages, LPS alone or in combination with cytokine like IFNγ, and calcium ionophore A23187 are the best-characterized stimuli to induce the transcription of genes encoding pro-inflammatory proteins, resulting in cytokine release and synthesis of enzymes such as COX-2 and 5-LOX, respectively. The inducible isoform COX-2 and 5-LOX would be responsible for the high prostanoid generation during inflammatory responses [31]. In the current investigation *M. indica* extract and mangiferin inhibited the release of PGE_2 and LTB_4 from calcium ionophore- and LPS–IFNγ-stimulated macrophages.

On the other hand, these our results demonstrated the inhibitory activity of *M. indica* extract and mangiferin on human synovial secretory PLA_2. Secretory PLA_2s exert proinflammatory effects in some animal models [32]. This group of enzymes has been found in a number of pathological fluids associated with inflammatory conditions [33] and inhibitions of these enzymatic activities have been reported to inhibit the development of inflammatory responses [15].

Taken together our current findings, it is evident that both COX and LOX pathways of arachidonate metabolism are inhibited, suggesting that *M. indica* extract might be classified as a dual inhibitor. Moreover, the step proceeding AA release, which is catalyzed by PLA_2 was also a target of *M. indica* extract with a potent effect.

We concluded that those compounds present in this natural product interact in vitro with the eicosanoid system and phospholipase level. Its main component mangiferin could be responsible, in part, of this activity. In vivo, this effect is potentiated with the inhibitory action on TNFα serum level and MPO activity.

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