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Anti-allergic properties of *Mangifera indica* L. extract (Vimang) and contribution of its glucosylxanthone mangiferin

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Abstract

Vimang is the brand name of formulations containing an extract of *Mangifera indica* L., ethnopharmacologically used in Cuba for the treatment of some immunopathological disorders, including bronchial asthma, atopic dermatitis and other allergic diseases. However, the effects of Vimang on allergic response have not been reported until now. In this study, the effects of Vimang and mangiferin, a C-glucosylxanthone isolated from the extract, on different parameters of allergic response are reported. Vimang and mangiferin showed a significant dose-dependent inhibition of IgE production in mice and anaphylaxis reaction in rats, histamine-induced vascular permeability and the histamine release induced by compound 48/80 from rat mast cells, and of lymphocyte proliferative response as evidence of the reduction of the amount of B and T lymphocytes able to contribute to allergic response. In these experiments, ketotifen, promethazine and disodium cromoglicate were used as reference drugs. Furthermore, we demonstrated that Vimang had an effect on an in-vivo model of inflammatory allergy mediated by mast cells. These results constitute the first report of the anti-allergic properties of Vimang on allergic models, as well as suggesting that this natural extract could be successfully used in the treatment of allergic disorders. Mangiferin, the major compound of Vimang, contributes to the anti-allergic effects of the extract.

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Introduction

Allergy is a major health problem in most modern societies, and the prevalence of certain allergic disorders has been enhanced in many countries during the past few decades (Woolcock 2000). This phenomenon is known to be more relevant for type I allergic diseases (anaphylactic type), such as bronchial asthma, allergic rhinoconjunctivitis (namely hay fever) and atopic dermatitis, but it has not been seen in other types of allergic diseases, namely type II or cytotoxic, type III or mediated by immunocomplex, or type IV or delayed type reaction (Ring et al 2001).

Mast cells represent one of the most important cells in the type I allergic response, behaving as highly specialized secretory cells widely distributed throughout the tissues, particularly in proximity to blood vessels, nerves and epithelial surfaces. They are known to be activated through the interaction of their surface receptors with specific molecules. These interactions initiate a series of biochemical events resulting in the release of biologically active mediators that cause allergic reaction. The major mechanism for the stimulation of these cells is the interaction of antigen (allergen) with IgE bound to its high-affinity receptor (FcRI), on the cell surface. Cross-linkage of FcRI results in activation of the cell and the initiation of signal transduction cascade that leads to the release of two general types of mediators. The first are preformed mediators, such as TNFα, IL-4, histamine, heparin, serotonin, kinins and proteases, which are released immediately upon activation of the mast cell. The second are newly synthesized mediators, such as IL-1, IL-12, IL-13, IL-15, IL-16, TNFα and chemokines, as well as products of arachidonic acid metabolism. All these molecules have important effects on inflammatory cell activation and recruitment, smooth muscle

contraction, vasodilatation, increase of vascular permeability, bronchoconstriction, mucus secretion and other events associated with allergic response (Wedemeyer et al 2000; Robbie-Ryan & Brown 2002; Bradding 2003).

There are other important groups of cells involved in the allergic response (e.g., basophils, eosinophils, B and Th2 lymphocytes, neutrophils and others): basophils are cells with similar function to those of mast cells; eosinophils are rapidly recruited to the tissues to release important mediators; B and Th2 lymphocytes, respectively, produce IgE and diverse cytokines; and neutrophils produce lipid mediators, reactive oxygen species and proteases (Nizar & Kelly 2002).

Vimang is the brand name of formulations from an aqueous extract of the stem bark of selected varieties of *Mangifera indica* L. (Anacardiaceae) traditionally used in Cuba for its anti-inflammatory, analgesic, antioxidant and immunomodulatory properties, all of which have been demonstrated in scientific research (Sánchez et al 2000; Garrido et al 2001; García et al 2002; Leiro et al 2004). Several authors have reported alternative pharmacological actions of extracts of *Mangifera indica* L., including spasmolytic, anti-amoebic, antimicrobial and antipyretic effects (Das et al 1989; Awe et al 1998; Frame et al 1998; Tona et al 2000).

The chemical composition of this extract has been characterized by chromatographic (planar, liquid and gas) methods, mass spectrometry and UV/VIS spectrophotometry. A phytochemical investigation of mango stem bark extract has led to the isolation of seven phenolic constituents – gallic acid, 3,4-dihydroxy benzoic acid, gallic acid methyl ester, gallic acid propyl ester, mangiferin, (+)-catechin, (−)-epicatechin, benzoic acid and benzoic acid propyl ester. The extract also contains triterpenes, phytosterols, fatty acids and microelements. Quantitative analysis of the compounds has been performed by HPLC, and mangiferin was found to be the predominant component (Nuñez-Sellés et al 2002). Mangiferin is a C-glucosylxanthone molecule with antiviral, anti-tumour, anti-diabetic and antioxidant activity (Zheng & Lu 1990; Ichiki et al 1998; Scartezzini & Speroni 2000; Yoosook et al 2000; Miura et al 2001; Yoshimi et al 2001).

The effects of Vimang and mangiferin on allergic response have been previously studied only in a parasitic model (García et al 2003), but their effects on known allergic models and mast-cell-mediated allergic inflammation remain unknown. Therefore, our aim was to study the anti-allergic effects of Vimang and mangiferin using animal models of allergy and, specifically, to find out their effects on IgE production and anaphylaxis, on histamine-induced vascular permeability and the histamine release from mast cells, on lymphocyte proliferative response, and on an in-vivo model of inflammatory allergy mediated by mast cells.

Materials and Methods

Animals

Female Balb/c mice, 18–20 g, and male Wistar rats, 250–300 g, were purchased from the National Center for Laboratory Animal Production (CENPALAB, Havana, Cuba). They were housed in Macrolon cages (Panlab, Barcelona, Spain), in a standard bio-clean animal room, and kept under a 12-h light–dark cycle at 22–24°C. The animals had free access to food and tap water, and were allowed to acclimatize for one week before the experiments. All experiments were carried out 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. All experimental protocols were approved by the Quality Department of the Center of Pharmaceutical Chemistry (Havana, Cuba).

Reagents

Ovoalbumin (OVA), compound 48/80, ketotifen, Evan's blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT), histamine, indometacin, sodium cromoglicate, Histopaque and other reagents were obtained from Sigma (St Louis, MO, USA). Orthophthalaldehyde and formamide were obtained from Merck (Barcelona, Spain).

Preparation of extract of *Mangifera indica* L. (Vimang) and isolation of mangiferin

M. indica L. 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 Environment, Havana, Cuba. Stem bark extract of *M. indica* was prepared by decoction with water for 1 h and then it was concentrated by evaporation and spray-dried to obtain a fine homogeneous brown powder with a particle size of 30–60 μm (Nuñez-Sellés et al 2002). The chemical composition of the extract is described above.

We also studied the effects of mangiferin, a C-glucosylxanthone (1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside) that is present in significant quantities in Vimang. It was supplied by the Laboratory of Analytical Chemistry, Center of Pharmaceutical Chemistry (Cuba) and had been isolated from *M. indica* stem bark standardized extract by extraction with methanol, and a yellow powder was obtained with 90% of purity determined by HPLC (Nuñez-Sellés et al 2002).

The lot used in this study was analysed in the Quality Department of the Pharmaceutical Chemistry Center (Havana, Cuba) and this analysis showed that the Vimang lot had the following content: moisture $<10\%$, water-soluble substances $>50\%$, total phenol (in anhydrous base) $>30\%$ and mangiferin $>10\%$, according to the quality specification established.

Effect of Vimang and mangiferin on IgE levels

Groups of six Balb/c mice were conformed and immunized by intraperitoneal administration of 100 μg of OVA prepared in adjuvant solution (Ovary 1986). After one week, the mice were boosted with the same amount of OVA. For 21 days after the first immunization, the mice received oral treatment with Vimang (50, 100 or 250 mg kg^{-1}), mangiferin (50 mg kg^{-1}) or ketotifen (3 mg kg^{-1}). When the treatment was completed, the mice were bled via the retro-orbital plexus and the serum was stored at −70°C until use. The antibody titers of IgE serum were measured by performing 48-hour passive cutaneous

anaphylaxis reactions (PCA) of male Wistar rats. Briefly, 0.1 mL of serial dilutions (1/64, 1/128, 1/264, and 1/512) of mice IgE serum was injected intradermally (in duplicate) into the shaved skin of male Wistar rats. After 48 h, antigen challenge was performed by intravenous injection of 1 mL of OVA 0.1% dissolved in Evan's blue 1% in saline solution. The rats were sacrificed 30 min after the challenge, and the skin was removed for the determination of extravased dye at each reaction site. Dye extraction for the determination of the reaction strength was performed with formamide as solvent (Katayama et al 1981). A calibration curve to Evan's blue (10–0.05 $\mu g\,mL^{-1}$) was carried out.

Effect of Vimang and mangiferin on anaphylactic reaction

Wistar rats (8 per group) were treated orally for 21 days with Vimang (50, 100 or 250 $mg\,kg^{-1}$) or mangiferin (50 $mg\,kg^{-1}$). They were then sensitized, by intradermal injection into the shaved skin, with serial dilutions (1/32, 1/64 and 1/128, in triplicate) of mice antiserum with high titres of IgE (previously obtained in control groups of mice). An anaphylactic reaction was induced 48 h later by intravenous injection of 1 mL of OVA 0.1% dissolved in Evan's blue 1% in saline solution. Five minutes before that last step, one group of rats received disodium cromoglicate (3 $mg\,kg^{-1}$) as reference drug. Dye extraction for the determination of the reaction strength was performed according to Katayama et al (1981). The amount of dye extravasated was considered proportional to the intensity of anaphylactic reaction.

Effect of Vimang and mangiferin on histamine-induced cutaneous reactions in rats

Male Wistar rats (8 per group) were treated orally for 7 days with Vimang (50, 100 or 250 $mg\,kg^{-1}$), mangiferin (50 $mg\,kg^{-1}$) or promethazine (25 $mg\,kg^{-1}$). Then, they were injected intravenously with 1 mL of Evan's blue 1% in saline solution, and immediately received 50 μg/site of histamine intradermally into 4 sites of the shaved back, and another two sites received saline. The rats were sacrificed 30 min later, and the reaction sites were excised for measurement of extravased dye (Abe et al 1994). The amount of extravased dye was determined according to Katayama's method (Katayama et al 1981).

Effect of Vimang and mangiferin on compound 48/80-induced histamine release from rat peritoneal mast cells

Male Wistar rats were sacrificed by stunning and cutting the neck blood vessels. The peritoneal cavity was washed with 20 mL of Tyrode solution (composition in mM: NaCl 137; $NaHCO_3$ 12; NaH_2PO_4 0.3; KCl 2.7; $MgCl_2$ 1; $CaCl_2$ 1.8; dextrose 5.6). The washing from rats was collected, pooled and kept on ice until use. The washings were centrifuged at 250 *g* for 5 min. The supernatant was discarded and the cell pellet was resuspended in a cold Tyrode solution. Mast cells were separated from the major components of rat peritoneal cells, according to the method described by Yurt et al (1977). Briefly, peritoneal cells suspended in 1 mL Tyrode solution were layered on 2.0 mL of 22.5% (w/v) metrizamide (density 1.120 $g\,mL^{-1}$) and centrifuged at room temperature for 15 min at 400 *g*. The cells remaining at the buffer–metrizamide interface were aspirated and discarded, and the cell pellet was washed and resuspended in 1 mL of Tyrode. More than 95% of cells were mast cells, assessed by toluidine blue staining. More than 96% of the cells were viable as judged by the trypan blue uptake.

The cell suspension (1-mL volumes in duplicate) was pre-incubated at 37°C for 10 min with three different concentrations of Vimang or mangiferin (50, 100 or 250 $\mu g\,mL^{-1}$), or disodium cromoglicate (100 μM), and stimulated for a further 5 min with compound 48/80 (1 $\mu g\,mL^{-1}$). Placing the tubes in an ice bath stopped the reaction. Each sample was centrifuged at 250 g for 5 min, the supernatant was decanted (for determination of release histamine) and the pellets resuspended in 1 mL of Tyrode solution and then boiled for 10 min to release residual histamine (Remírez et al 2002). Released and residual histamine was measured fluorimetrically at 440 nm (excitation at 360 nm) in a spectrofluorometer (Shore et al 1959). The results were expressed as the amount of histamine released in each condition. A calibration curve to histamine (0.1–100 μM) was carried out.

Effect of Vimang and mangiferin on OVA-induced paw oedema and on lymphocyte-specific proliferation

Seven groups of Balb/c mice (8 per group) were conformed. Six of these groups were actively immunized by seven intraperitoneal injections of 10 μg of OVA in 0.5 mL of saline solution on alternate days. The other group of mice received injections only of saline solution. Three weeks after the last injection, and for seven days, the mice received treatment with Vimang (50, 100 or 250 $mg\,kg^{-1}$), mangiferin (50 $mg\,kg^{-1}$) or indometacin (5 $mg\,kg^{-1}$).

Induction of inflammatory allergic response by OVA-induced paw oedema

At the end of treatment, 10 μg/paw of OVA was injected into the subplantar area of the right hind paw, and saline solution was injected into the left hind paw. One hour later, the size of the oedema was assessed by measuring the weight of the right and left hind paw in each mouse. The mice 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 in weight of the right hind paw versus the left hind paw, and was called oedema index (Mi-Sun et al 2003).

Antigen-specific lymphocyte proliferative response

Lymphocytes were obtained from the spleens of mice killed previously and were purified by Histopaque density gradient centrifugation. The cellular suspension was adjusted to a working dilution of 2×10^6 cells/mL. Lymphocytes were cultured in flat-bottom 96-well microtitre plates (Corning, USA) in the presence or absence of OVA 10 $\mu g\,mL^{-1}$ for 72 h,

at 37°C under 5% CO_2. The lymphocyte proliferation was assessed by MTT assay (Youl et al 1999). The group treated with indometacin were not evaluated in this test.

Statistics

Results were expressed as the means ± standard error of means (s.e.m.) of three independent experiments. The significant differences between the groups were determined by the Kruskal–Wallis non-parametric test, and when significant differences were obtained between groups, the Dunn's post-test was used. For all tests, $P < 0.05$ were considered significant.

Regression analysis was used to calculate the effective dose 50 (ED50), defined as the dosage of the extract necessary to produce a 50% effect. In a similar manner, the inhibitory concentration 50 (IC50) for the in-vitro experiments was calculated.

Results

Effect of Vimang and mangiferin on IgE levels

The effects of Vimang and mangiferin on IgE production mice were evaluated through PCA reaction in rats sensitized with the mice antiserum treated with Vimang and mangiferin (Table 1). Pretreatment for 21 days with Vimang (100 and 250 mg kg^{-1}) and mangiferin (50 mg kg^{-1}) significantly reduced the anti-OVA IgE levels in mice, measured as the amount of Evan's blue extravased. Ketotifen, used as a reference drug, also reduced the specific IgE levels.

Effect of Vimang and mangiferin on anaphylactic response

The effects of pretreatment for 21 days with Vimang and mangiferin on the anaphylactic response induced in rats through sensitization with high-IgE-titre serum have been evaluated. Oral treatment with 100 and 250 mg kg^{-1} of Vimang and 50 mg kg^{-1} of mangiferin inhibited the appearance of cutaneous anaphylaxis induced by OVA in sensitized rats. Intravenous administration of 3 mg kg^{-1} disodium cromoglicate before the antigen challenge produced significant inhibition of the reaction (Table 2).

Effect of Vimang and mangiferin on histamine-induced cutaneous reactions in rats

In this experiment, the effects of pretreatment with different doses of Vimang and mangiferin on histamine-induced cutaneous reactions in rats were evaluated. Histamine provoked an

Table 1 Effect of treatment with Vimang, mangiferin and ketotifen on IgE levels in mice

Treatment	Dose (mg kg^{-1})	Evan's blue extravased (μg/site)			
		Dilution of serum			
		1/64	1/128	1/256	1/512
Control	—	17.2 ± 0.2	10.9 ± 0.03	6.2 ± 0.02	2.8 ± 0.14
Vimang	50	16.4 ± 0.21	10.8 ± 0.52	6.9 ± 0.08	2.8 ± 0.04
	100	14.1 ± 0.9*	8.5 ± 0.56*	3.5 ± 0.05*	0.8 ± 0.01*
	250	13.3 ± 0.33*	7.8 ± 0.02*	3.2 ± 0.18*	0.5 ± 0.05*
Mangiferin	50	8.3 ± 0.29*	4.7 ± 0.06*	3.6 ± 0.06*	1.5 ± 0.08*
Ketotifen	3	10.4 ± 0.33*	5.2 ± 0.48*	2.3 ± 0.25*	0.6 ± 0.04*

IgE levels were determined by PCA in rats and expressed as μg of Evan's blue extravased/site. The results are the means ± s.e.m. of three independent experiments. *$P < 0.05$, compared with control group.

Table 2 Effect of treatment with Vimang, mangiferin and sodium cromoglicate on anaphylactic response in rats

Treatment	Dose (mg kg^{-1})	Evan's blue extravased (μg/site)		
		Dilution of serum		
		1/32	1/64	1/128
Control	—	33.5 ± 4.7	16.5 ± 2.5	12.7 ± 1.5
Vimang	50	25.8 ± 2.8**	14.5 ± 1.5	8.2 ± 1.5
	100	19.4 ± 4.5**	11.8 ± 3.9*	6.6 ± 1.6*
	250	16.5 ± 2.6**	9.2 ± 2.9*	5.9 ± 0.6*
Mangiferin	50	26.7 ± 2.3**	11.6 ± 1.8*	8.3 ± 1.3*
Disodium cromoglicate	3	18.4 ± 2.6**	9.3 ± 2.72*	5.6 ± 1.40*

Data are expressed as μg of Evan's blue extravased/site. The results are the means ± s.e.m. of three independent experiments. *$P < 0.05$, **$P < 0.01$, compared with control group.

Table 3 Effect of Vimang, mangiferin and promethazine on histamine-induced cutaneous reactions in rats

Treatment	Dose (mg kg^{-1})	Evan's blue extravased (μg/site)	% Inhibition of cutaneous reaction
Control	—	23.12 ± 1.11	—
Vimang	50	14.58 ± 3.51*	37
	100	10.44 ± 1.33**	55
	250	7.88 ± 2.76**	66
Mangiferin	50	8.49 ± 2.31**	63
Promethazine	25	0.81 ± 0.19***	97

Data are expressed as μg of Evan's blue extravased/site and % of inhibition. The results are the means ± s.e.m. of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with control group.

increase in vascular permeability that conduced to the extravasation of Evan's blue administered intravenously. As shown in Table 3, the cutaneous reaction induced by histamine in rats was found to be maximal in the control group. This reaction was inhibited significantly by Vimang (ED50 = 84.9 mg kg^{-1}) and mangiferin when they were administered over the previous seven days. The maximal inhibitory effect shown by Vimang and mangiferin was 66 and 63%, respectively. Promethazine, a known antihistaminic type I, showed a 97% inhibition on histamine-induced cutaneous reactions (Table 3).

Effect of Vimang and mangiferin on compound 48/80-induced histamine release from rat peritoneal mast cells

The release of histamine from mast cells has been evaluated with compound 48/80, a known mast cell degranulator. In this experiment, the histamine spontaneously released from rat peritoneal mast cells was 3%, and the maximal release induced by compound 48/80 was 53%. Vimang and mangiferin inhibited markedly the histamine release from peritoneal mast cells following a 10-min preincubation (Figure 1). The IC50 of Vimang and mangiferin was 51.5 and 60.9 μg mL^{-1}, respectively.

Effect of Vimang and mangiferin on OVA-induced paw oedema and lymphocyte proliferation specific to OVA

Paw oedema induced by subplantar injection of OVA in immunized mice and treated with Vimang and mangiferin was evaluated. One hour after subplantar injection of OVA, the oedema was significantly higher in the control group. Mice pretreated with Vimang and indometacin showed markedly less oedema than the control group (Table 4). The maximal inhibition was 56% for Vimang 250 mg kg^{-1} and 63% for indometacin 5 mg kg^{-1}. The ED50 for Vimang was 192 mg kg^{-1}. Mangiferin did not show inhibitory effects on this parameter at the evaluated dose.

Moreover, the treatment with Vimang and mangiferin had inhibitory effects on the lymphocyte proliferative response to OVA stimulation. The results obtained with Vimang (100 and 250 mg kg^{-1}) and mangiferin (50 mg kg^{-1}) were similar to the non-immunized mice group (Figure 2).

Discussion

In this study, the anti-allergic effects of Vimang, an aqueous extract of *Mangifera indica* L., and mangiferin were investigated using animal models of allergy.

IgE is essential for the development of allergic reactions, and some studies have also shown the local production of IgE in allergic airway diseases (Smurthwaite & Durham 2002). In this study, we performed PCA reaction tests, which are known as an animal model for type I allergic reaction. Vimang and mangiferin reduced IgE levels in OVA-immunized mice, and also reduced the passive anaphylactic reaction in rats.

Mast cells are likely to play a significant role in the pathophysiology of asthma and allergic diseases through their immunomediatory secretory activity as a response to their activation by allergens and other secretory stimuli. Mast cells synthesize and secrete a number of cytokines, which regulate both IgE synthesis and the development of eosinophilic inflammation (Bradding 1996).

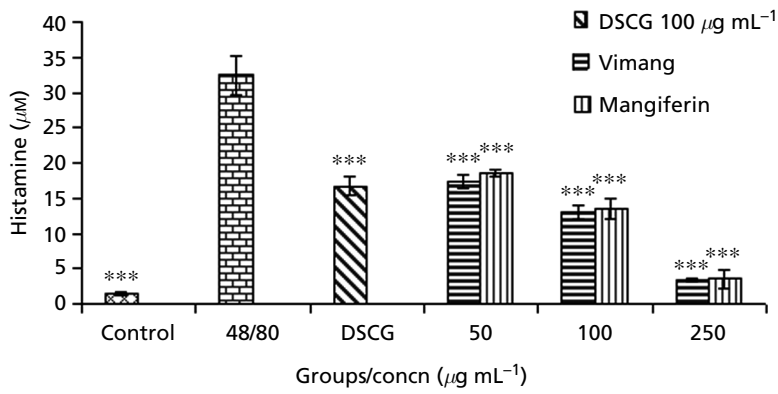

Figure 1 Effect of Vimang, mangiferin and sodium cromoglicate (disodium cromoglicate, DSCG) on compound 48/80-induced histamine release from rat peritoneal mast cells, determined by fluorimetric method. The results are the means ± s.e.m. of three independent experiments. ***$P < 0.001$, compared with 48/80 group.

Table 4 Effects of Vimang, mangiferin and indometacin on mast cell-derived paw oedema induced by OVA in immunized mice

Treatment	Dose (mg kg^{-1})	Oedema index (g)	% Inhibition of allergic inflammation
NI	—	0.012 ± 0.008	—
Control	—	0.05 ± 0.007	—
Vimang	50	0.039 ± 0.002*	28
	100	0.033 ± 0.003**	44
	250	0.028 ± 0.004***	56
Mangiferin	50	0.046 ± 0.006	11
Indomethacin	5	0.026 ± 0.002***	63

Data are expressed as oedema index (mg) and % of inhibition of allergic inflammation. NI, non-immunized group. The results are the means ± s.e.m. of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with control group.

To assess the effects of Vimang and mangiferin on mast cell activity, we evaluated the histamine-induced vascular permeability and the histamine release from mast cells. The histamine-induced cutaneous reaction in rats was clearly inhibited in a dose-dependent fashion by Vimang and mangiferin when they were administered orally for the preceding seven days. Also, they reduced compound 48/80-induced histamine release from mast cells. These results demonstrate that Vimang and mangiferin inhibit the mast cell-dependent anaphylactic reaction, and indicate that this activity could be due to the inhibition of histamine release from mast cells.

Previous studies have demonstrated the antioxidant effects of Vimang (Sánchez et al 2000, 2001). Studies involving blood cells have indicated that free radicals can induce histamine release by a calcium-independent process (Di Bello et al 1998). If free radicals are important in allergic response, enhancement of the antioxidant defences would be expected to have beneficial effects in these diseases, and treatment with Vimang could contribute to it.

We evaluated the effects of Vimang and mangiferin on the inflammatory allergic reaction induced by OVA in immunized mice. Repeated immunization of mice with OVA and then the induction of paw oedema with the same allergen resulted in the development of an inflammatory allergy mediated by mast cells, characterized by activation of proteinase-activated receptor-2 (PAR-2), a receptor for mast cell tryptase, granulocytes infiltration and increased vascular permeability (Vergnolle et al 1999). The treatment with Vimang reduced significantly the paw oedema induced by OVA, which provided evidence of its inhibitory effects on allergic inflammation, but mangiferin did not show inhibitory effects, suggesting that others component of Vimang could contribute to its effect.

The effects of Vimang and mangiferin on antigen-specific lymphocyte proliferation have been also evaluated. When lymphocytes of mice immunized with OVA are cultured in-vitro in the presence of OVA, the specific clones that were activated during in-vivo immunization should respond to antigen through an increase in the number of specific lymphocytes, a process known as proliferation. The treatment with Vimang and mangiferin demonstrated a reduction in the proliferative lymphocyte response, as evidence of the reduction of B and T lymphocytes able to contribute to the allergic response. The inhibition of lymphocyte proliferation could explain the inhibitory effects of Vimang and mangiferin on IgE production, because, they reduce the number of B cells able to produce anti-OVA specific IgE.

The allergic response is an important component of the inflammatory response. There are some reports of the anti-inflammatory effects of Vimang, and the contribution of mangiferin (Garrido et al 2001, 2004). Previously, we also demonstrated the inhibitory effects of Vimang and mangiferin on mRNA iNOS, COX-2, TNFα, IL-1β, GM-CSF and NFκB expression (Leiro et al 2004). These antecedents, and the results presented in this paper, could explain the contribution of anti-inflammatory effects of Vimang and mangiferin to allergic response.

Polyphenols are known to inhibit basophil histamine release and neutrophil betaglucuronidase release, and thereby possess in-vivo anti-allergic activity (Cheong et al 1998). Taking into account our results, the anti-allergic activity of

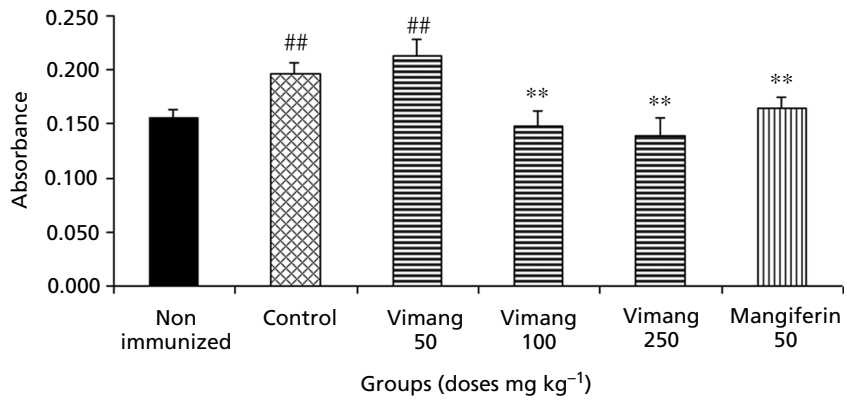

Figure 2 Effect of Vimang and mangiferin on lymphocyte proliferative response to OVA in immunized mice, determined by MTT assay. The results are the means ± s.e.m. of three independent experiments. **$P < 0.01$, compared with control group; ##$P < 0.01$, compared with non-immunized group.

Vimang could be at least partially attributable to mangiferin, its major compound.

It is now accepted that type Th2 helper cytokines play an essential role in the pathogenesis of allergic inflammation, including atopy and asthma. IL-4, IL-5, IL-9 and IL-13 are Th2 cytokines and these cytokines can induce the chemotaxis of immune cells, mucus hypersecretion and expression of extracellular matrix proteins. Therefore, the effect of Vimang on the regulation of Th2 cytokines needs to be investigated in further studies.

Conclusion

Our results indicate that Vimang has anti-allergic properties, as demonstrated by the inhibition of IgE production and anaphylaxis, inhibition of histamine-induced vascular permeability and the histamine release from mast cells, and reduction in proliferative lymphocyte response. Also, Vimang reduces an in-vivo model of inflammatory allergy mediated by mast cells. In this study, we have demonstrated that mangiferin contributes to the anti-allergic properties of Vimang.

This is the first report of the anti-allergic properties of Vimang, and suggests that it could be used in the treatment of allergic disorders, such as rhinitis, atopic dermatitis and bronchial asthma.

References

Abe, T., Omata, T., Yoshida, K., Natsumara, T., Ikeda, Y., Segawa, Y., Matsuda, K., Nagai, H. (1994) Antiallergic effect of ZRC-2060: antihistaminic action. *Jpn. J. Pharmacol*. **66**: 87–94

Awe, S. O., Olajide, O. A., Oladiran, O., Makinde, J. M. (1998) Antiplasmodial and antipyretic screening of *Mangifera indica* extract. *Phytother. Res*. **12**: 437–438

Bradding, P. (1996) Human mast cell cytokines. *Clin. Exp. Allergy* **26**: 13–19

Bradding, P. (2003) The role of the mast cell in asthma: a reassessment. *Curr. Opin. Allergy Clin. Immunol*. **3**: 45–50

Cheong, H., Ryu, S. Y., Oak, M. H., Cheori, S. H., Yoo, G. S., Kim, K. M. (1998) Studies of structure activity relationship of flavonoids for the antiallergic actions. *Arch. Pharmacol. Res*. **21**: 478–480

Das, P. C., Das, A., Mandal, S. (1989) Anti-inflammatory and antimicrobial activities of the seed kernel of *Mangifera indica*. *Fitoterapia* **LX**: 235–240

Di Bello, M. G., Masini, E., Ioannides, C., Fomusi, J., Raspanti, S., Bani, T., Mannaioni, P. F. (1998) Histamine release from rat mast cells induced by the metabolic activation of drugs of abuse into free radicals. *Inflamm. Res*. **47**: 122–130

Frame, A. D., Rios-Olivares, E., de Jesús, L., Ortiz, D., Pagan, J., Méndez S. (1998) Plants from Puerto Rico with anti-*Mycobacterium tuberculosis* properties. *P. R. Health Sci. J*. **17**: 243–252

García, D., Delgado, R., Ubeira, F. M., Leiro, J. (2002) Modulation of rat macrophage function by the *Mangifera indica* L. extract Vimang and mangiferin. *Int. Immunopharmacol*. **2**: 797–806

García, D., Escalante, M., Delgado, R., Ubeira, F. M., Leiro, J. (2003) Antihelminthic and antiallergic activities of *Mangifera indica* L. stem bark components Vimang and mangiferin. *Phytother. Res*. **17**: 1203–1208

Garrido, G., González, D., Delporte, C., Backhouse, N., Quintero, G., Núñez-Séllez, A., Morales, M. (2001) Analgesic and anti-inflammatory effects of *Mangifera indica* L. extract (Vimang). *Phytother. Res*. **15**: 18–21

Garrido, G., Delgado, R,. Lemus, Y., Rodríguez, J., García, D., Núñez-Séllez, A. (2004) Protection against septic shock and suppression of tumor necrosis factor alpha and nitric oxide production on macrophages and microglia by a standard aqueous extract of *Mangifera indica* L. (Vimang). Role of mangiferin isolated from the extract. *Pharmacol. Res*. **50**: 165–172

Ichiki, H., Miura, T., Kubo, M., Ishihara, E., Komatsu, Y., Tanigawa, K., Ojada, M. (1998) New antidiabetic compounds, mangiferin and its glucoside. *Biol. Pharm. Bull*. **21**: 1389–1390

Katayama, S., Akimoto, N., Morimotio, T., Karoh, Y. (1981) Anti-allergic effect of Azelastine hydrochloride on immediate type hypersensitivity reactions *in vivo* and *in vitro*. *Drug Res*. **8**: 1196–1203

Leiro, J., García, D., Arranz, J. A., Delgado, R., Sanmartín, M. L., Orallo, F. (2004) An Anacardeacea preparation reduces the expression of inflammation-related genes in murine macrophages. *Int. Immunopharmacol*. **4**: 991–1003

Mi-Sun, K., Ho-Jeong, N., Seung-Woo, H., JonSik, J., Un-Yong, S., Eon-Jeong, L., Bong-Keun, S., Seung-Heon, H., Hyung-Min, K. (2003) *Forsythia fructus* inhibits the mast cell mediates allergic inflammatory reactions. *Inflammation* **27**: 129–135

Miura, T., Ichiki, H., Hashimoto, I., Iwamoto, N., Kato, M., Kubo, M., Ishihara, E., Komatsu, Y., Okada, M., Ishida, T., Tanigawa, K. (2001) Antidiabetic activity of a xanthone compound, mangiferin. *Phytomedicine* **8**: 85–87

Nizar, J., Kelly, E. (2002) Pathogenesis of asthma. *Med. Clin. North Am*. **86**: 925–936

Núñez-Sellés, A. J., Vélez-Castro, H. T., Aguero-Aguero, J., Gonzalez-Gonzalez, J., Naddeo, F., De Simone, F., Rastrelli, L. (2002) Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. *J. Agric. Food Chem*. **50**: 762–766

Ovary, Z. (1986) Passive cutaneous anaphylaxis. In: Weir, D. M. (ed.) *Handbook of experimental immunology*. 4th edn, Vol. 1, Blackwell Scientific Publications, Oxford, pp 33.1–33.8

Remírez, D., Ledón, N., González, R. (2002) Role of histamine in the inhibitory effects of phycocyanin in experimental models of allergic inflammatory response. *Med. Inflamm*. **11**: 81–85

Ring, J., Kramer, U., Schfer, T., Behrendt, H. (2001) Why are allergies increasing? *Curr. Opin. Immunol*. **13**: 701–708

Robbie-Ryan, M., Brown, M. (2002) The role of mast cells in allergy and autoimmunity. *Curr. Opin. Immunol*. **14**: 728–733

Sánchez, G. M., Delgado, R., Pérez, G., Garrido, G., Núñez-Séllez, A., León, O. S. (2000) Evaluation of the *in vitro* antioxidant activity of *Mangifera indica* L. extract (Vimang). *Phytother. Res*. **14**: 424–427

Sánchez, G. M., Candelario-Jalil, E., Giuliani, A., León, O. S., Sam, S. R., Delgado, R., Núnez-Sellés, A. J. (2001) *Mangifera indica* L. extract (Vimang) reduces ischemia-induced neuronal loss and oxidative damage in the gerbil brain. *Free Radic. Res*. **35**: 465–473

Scartezzini, P., Speroni, E. (2000) Review on some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol*. **71**: 23–33

Shore, P. A., Burkhalter, A., Cohn, V. N. (1959) A method for the fluorimetric assay of histamine in tissues. *J. Pharmacol. Exp. Ther*. **127**: 182–186

Smurthwaite, L., Durham, S. R. (2002) Local IgE synthesis in allergic rhinitis and asthma. *Curr. Allergy Asthma Rep*. **2**: 231–239

Tona, L., Kambu, K., Ngimbi, N., Mesia, K., Penge, O., Lusakibanza, M., Cimanga, K., De Bruyne, T., Apers, S., Totte, J., Pieters, L., Vlietinck, A. J. (2000) Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. *Phytomedicine* **7**: 31–38

Vergnolle, N., Hollenberg, M. D., Sharkey, K. A., Wallace, J. L. (1999) Characterization of the inflammatory response to proteinase-activated receptor-2 (PAR2) activating peptides in the rat paw. *Br. J. Pharmacol*. **127**: 1083–1090

Wedemeyer, J., Tsai, M., Galli, S. (2000) Roles of mast cells and basophils in innate and acquired immunity. *Curr. Opin. Immunol*. **12**: 624–631

Woolcock, A. J. (2000) The burden of asthma. *ACI Int. Suppl*. **1**: 53–56

Yoosook, C., Bunyapraphatsara, N., Boonyakiat, Y., Kantasuk, C. (2000) Anti-*Herpes simplex* virus activities of crude water extracts of Thai medicinal plants. *Phytomedicine* **6**: 411–419

Yoshimi, N., Matsunaga, K., Katayama, M., Yamada, Y., Kuno, T., Qiao, Z., Hara, A., Yamahara, J., Mori, H. (2001) The inhibitory effects of mangiferin, a naturally occurring glucosylxanthone, in bowel carcinogenesis of male F344 rats. *Cancer Lett*. **163**: 163–170

Youl, J., Ra, A., Sook, E., Up, K., Hwan, M. (1999) Immunomodulatory effect of arctigenin, a lignan compound, on tumour necrosis factor and nitric oxide production, and lymphocytes proliferation. *J. Pharm. Pharmacol*. **51**: 1267–1276

Yurt, R. W., Leid, R. W., Austen, K. F. (1977) Native heparin from rat peritoneal mast cells. *J. Biol. Chem*. **252**: 518–521

Zheng, M. S., Lu, Z. Y. (1990) Antiviral effect of mangiferin and isomangiferin on *Herpes simplex* virus. *Chin. Med. J*. **103**: 160–165