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Vimang (*Mangifera indica* L. extract) induces permeability transition in isolated mitochondria, closely reproducing the effect of mangiferin, Vimang's main component

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Abstract

Mitochondrial permeability transition (MPT) is a Ca^{2+}-dependent, cyclosporin A (CsA)-sensitive, non-selective inner membrane permeabilization process. It is often associated with apoptotic cell death, and is induced by a wide range of agents or conditions, usually involving reactive oxygen species (ROS). In this study, we demonstrated that *Mangifera indica* L. extract (Vimang), in the presence of 20 μM Ca^{2+}, induces MPT in isolated rat liver mitochondria, assessed as CsA-sensitive mitochondrial swelling, closely reproducing the same effect of mangiferin, the main component of the extract, as well as MPT-linked processes like oxidation of membrane protein thiols, mitochondrial membrane potential dissipation and Ca^{2+} release from organelles. The flavonoid catechin, the second main component of Vimang, also induces MPT, although to a lesser extent; the minor, but still representative Vimang extract components, gallic and benzoic acids, show respectively, low and high MPT inducing abilities. Nevertheless, following exposure to H_2O_2/horseradish peroxidase, the visible spectra of these compounds does not present the same changes previously reported for mangiferin. It is concluded that Vimang-induced MPT closely reproduces mangiferin effects, and proposed that this xanthone is the main agent responsible for the extract's MPT inducing ability, by the action on mitochondrial membrane protein thiols of products arising as a consequence of the mangiferin's antioxidant activity. While this effect would oppose the beneficial effect of Vimang's antioxidant activity, it could nevertheless benefit cells exposed to over-production of ROS as occurring in cancer cells, in which triggering of MPT-mediated apoptosis may represent an important defense mechanism to their host.

Keywords: Vimang; Mangiferin; Calcium; Mitochondrial permeability transition; Permeability transition pore; Protein thiol oxidation

1. Introduction

Antioxidants have the connotation of being healthy and safe. Many diseases are associated with oxidative stress [1,2] and the use of antioxidant-rich food or food

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supplements is often recommended to preserve or regain good health, causing high dosages of these compounds to be taken by large populational segments [3,4]. Yet, antioxidants may exhibit important adverse effects: after having exerted their presumed protective action, i.e., after having neutralized a reactive species, they may be converted into potentially harmful oxidation products [5,6].

Vimang is a mangiferin-enriched extract obtained from the stem bark of *Mangifera indica* L. (mango), which shows potent in vitro and in vivo anti-inflammatory and antioxidant activities [7–9]. It is now produced on an industrial scale in Cuba, where it is to be mainly used as a nutritional supplement. Chemical studies performed with this extract have led to the isolation and identification of phenolic acids, phenolic esters, flavan-3-ols and micronutrients such as selenium; its main component is likely to be the xanthone mangiferin [10,11] (Fig. 1).

In a previous study we demonstrated that mangiferin induces mitochondrial permeability transition (MPT), observing in addition a change in the spectrum of the xanthone exposed to H_2O_2/horseradish peroxidase, compatible with the formation of oxidation products of this compound [12]. In the present study, we addressed this issue on Vimang, focusing on mangiferin itself, but considering also three other representative components of the whole extract, namely gallic and benzoic acids, and the flavonoid catechin. It was concluded that Vimang induces MPT closely reproducing the same effect of mangiferin, and proposed that this xanthone is the main agent responsible for the extract's MPT inducing ability, by the action on mitochondrial membrane protein thiols of products arising as a consequence of mangiferin's antioxidant activity.

2. Materials and methods

2.1. Chemicals

Antimycin A, cyclosporin A (CsA), 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB), ethylene glycol-bis(β-aminoethyl ether)*N,N,N′,N′*-tetraacetic acid (EGTA), carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), *N*-ethylmaleimide (NEM), ruthenium red (RR), safranin, mangiferin, malate, pyruvate, α-ketoglutarate and 2-hydroxyethyl-1 piperazineethanesulfonic acid (HEPES) were obtained from Sigma Chemical Company (St. Louis, MO). All other reagents were commercial products of the highest purity grade available. The chemical composition of the extract has been characterized by chromatographic (planar, liquid and gas) methods, mass

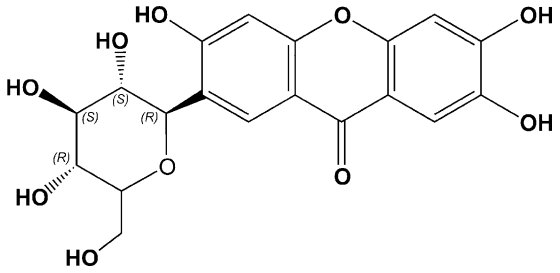

Fig. 1. Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-β-d-glucoside) structure.

spectrometry and UV/vis spectrophotometry [11]. The total polyphenol content of Vimang determined by the Folin–Ciocalteu method, and expressed as gallic acid equivalents, was 34/100 g dry weight. Mangiferin content was determined by HPLC methods and represents 16% of the powdered extract.

Stock solutions of Vimang were prepared by dissolving the solid extract in dimethyl sulfoxide (DMSO) at a concentration range of 1–10 mM mangiferin, equivalent to (2.6–26 mg/ml powder extract). Pure mangiferin was also dissolved in DMSO at 10 mM. Experiments were carried out at a 1/1000 (1–10 μM) dilution, to prevent solvent influence on the results. Controls experiments (without Vimang or mangiferin) contained 14.3 μM DMSO, which represents the solvent concentration in all Vimang/mangiferin samples.

2.2. Isolation of rat liver mitochondria (RLM) and standard incubation procedure

Mitochondria were isolated by conventional differential centrifugation of the liver of overnight fasted adult animals [13]. The tissue was homogenized in 250 mM sucrose, 1 mM EGTA, and 10 mM HEPES buffer (pH 7.2). The mitochondrial suspension was washed twice in the same 0.1 mM EGTA containing medium and the final pellet suspended in 250 mM sucrose (pH 7.2) to a final protein concentration of 80–100 mg/ml, as measured by the Biuret method, with bovine serum albumin used as protein standard [14]. All experiments were conducted within 3 h following mitochondrial isolation.

Experiments were carried out in a standard medium containing 125 mM sucrose, 65 mM KCl, 2 mM inorganic phosphate (Pi), 1 mM magnesium chloride, 10 mM HEPES buffer (pH 7.2) and a cocktail of NAD-linked substrates at 5 mM (malate, pyruvate, α-ketoglutarate). Other additions are indicated in the figure legends.

2.3. Mitochondrial respiration rates

Oxygen consumption in mitochondrial suspensions was polarographically determined with a Clark-type electrode (Yellow Springs Instruments Co.) in a 1.3-ml glass chamber equipped with a magnetic stirrer, at 28 °C. Respiration of mitochondria (1 mg of protein/ml) was initiated with a cocktail of NAD^+-linked substrates at 5 mM (malate, pyruvate, α-ketoglutarate) and phosphorylating (state III) respiration was initiated by addition of 200 nmol ADP/mg protein. Respiration rates are given in ng atoms oxygen/mg/min. O_2 consumption recordings allowed the calculation of the rates of state III respiration, state IV respiration, and respiratory control ratio (RCR = state III/state IV). The experimental approach was calibrated using the oxygen content of an air saturated medium of 425 ng atoms/ml, at 28 °C [15].

2.4. Mitochondrial swelling

Mitochondrial swelling was estimated from the decrease in absorbance of the mitochondrial suspension measured at 540 nm in a Hitachi U-3000 spectrophotometer equipped with magnetic stirring and temperature control (28 °C), essentially as previously described [16]. Mitochondria were incubated in the standard incubation medium at 0.5 mg of mitochondrial protein/ml.

2.5. Mitochondrial transmembrane electrical potential (ΔΨ)

The ΔΨ was estimated as fluorescence changes of safranin [17], recorded on a model F-4010 Hitachi spectrofluorimeter operating at excitation and emission wavelengths of 495 and 586 nm, respectively, with a slit width of 5 nm. Mitochondria (0.5 mg/ml) were incubated in 2 ml of standard medium supplemented with 5 μM safranin. After 30 s, 5 mM NAD-linked substrate cocktail was added. When ΔΨ was established, 20 μM Ca^{2+} was added to the medium and the times of ΔΨ collapses were compared. Relative changes in membrane potential were expressed in arbitrary fluorescence units and were not converted to potential values.

2.6. Ca^{2+} movement in mitochondria

Variations in free Ca^{2+} concentration were followed by measuring changes in the absorbance spectrum of the metallochromic indicator arsenazo III [18], using an SLM Aminco DW2000 spectrophotometer at the wavelength pair 675–685 nm, at 28 °C.

2.7. Mitochondrial membrane protein thiols content

Mitochondrial membrane protein thiol groups were determined by using DTNB (Ellman's reagent), as previously described [19]. Briefly, the mitochondrial suspension was incubated in the standard reaction medium, submitted to three subsequent freeze-thawing procedures to release matrix proteins and centrifuged for 2 min at 10,000 rpm in a 5415 C Eppendorf Centrifuge. The pellet was treated with 200 μl of 6.5% trichloroacetic acid and centrifuged at 10,000 rpm during 2 min in order to precipitate the protein. This procedure was repeated two times. The final pellet was suspended in 1 ml of a medium containing 0.5% sodium dodecyl sulfate, 0.5 mM EDTA, 0.5 M Tris, pH 8.3 and 100 μM 5,5′-dithio-bis (2-nitrobenzoic) acid. Absorption was measured at 412 nm, using glutathione for calibration. Under control conditions, total mitochondrial membrane protein thiol content was approximately 45 nmol/mg protein.

2.8. Statistics

Statistical analysis was performed by the unpaired Student's *t*-test and ANOVA where appropriate. All experiments were performed at least, in triplicate, using different mitochondrial preparations. Differences between groups were considered to be statistically significant at $P < 0.05$. Data are given as means ± S.D. ($n = 3$–5) or as a typical example.

3. Results

3.1. Effects of Vimang/mangiferin on mitochondrial respiratory parameters

Table 1 shows the effects elicited by Vimang/mangiferin plus Ca^{2+}, on mitochondrial respiratory parameters. Exposure of isolated rat liver mitochondria to Vimang or mangiferin, in the absence of Ca^{2+} (presence of EGTA), did not significantly affect mitochondrial respiration. Addition of 20 μM Ca^{2+} to mitochondria alone significantly reduced the respiratory control ratio (RCR); this effect was enhanced by the presence of Vimang or mangiferin due to both reduction of state III respiration rate by around 21%, and increase of state IV respiration rate by around 25%.

3.2. Vimang/mangiferin-induced swelling, protein thiol oxidation, ΔΨ dissipation and Ca^{2+} release in mitochondria

Fig. 2A shows that in the presence of 20 μM Ca^{2+}, increasing concentrations of Vimang induced progres-

Table 1
Effects of Vimang/mangiferin, and Ca^{2+}, on mitochondrial respiratory parameters

Conditions	State III	State IV	RCR
Control (200 μM EGTA)	158.00 ± 10.5	25.00 ± 6.2	6.32 ± 0.62
Vimang/mangiferin + 200 μM EGTA	156.00 ± 8.2	26.00 ± 4.4	6.0 ± 0.43
20 μM Ca^{2+}	140.3 ± 11.43[a]	27.5 ± 6.2	5.1 ± 0.52[a]
20 μM Ca^{2+} + 10 μM Vimang	110.5 ± 12.6[a,b,c]	32.5 ± 5.4[a,b,c]	3.4 ± 0.33[a,b,c]
20 μM Ca^{2+} + 10 μM Mangiferin	110.6 ± 10.6[a,b,c]	32.06 ± 3.8[a,b,c]	3.45 ± 0.63[a,b,c]

Experimental conditions are described in Section 2. Values represent averages of three experiments ±S.D., with different mitochondrial preparations.
[a] $P < 0.05$, non-paired Student's test compared with control.
[b] $P < 0.05$, non-paired Student's test compared with Vimang plus 200 μM EGTA.
[c] $P < 0,05$, non-paired Student's test compared with Ca^{2+} treatment.

sively larger swelling in mitochondria energized by NAD^+-linked substrates; 10 μM of mangiferin closely reproduced this effect; EGTA, added to eliminate the free Ca^{2+} present in the mitochondria preparation, and RR, added to block the Ca^{2+} uniporter-mediated movement of the cation into the mitochondrial matrix, completely inhibited mitochondrial swelling, indicating as postulated, a strict Ca^{2+}-dependence of the process. Fig. 2B shows that the amount of membrane protein thiol groups decreased significantly with increasing Vimang concentration in the presence of Ca^{2+}, and that the pattern of the concentration-response curves of this effect, closely followed the pattern of the curves describing the induction of mitochondrial swelling; 10 μM of mangiferin again closely reproduced this effect.

Besides inducing mitochondrial swelling, Vimang/mangiferin stimulated Ca^{2+}-mediated loss of $\Delta\Psi$ (Fig. 3A), and released Ca^{2+} pre-accumulated by mitochondria (Fig. 3B). All these effects were almost completely inhibited by the classical MPT inhibitors CsA and NEM, indicating that they are intrinsic to the MPT process.

Table 2
Effects of MPT inhibitors on Vimang-induced oxidation of mitochondrial membrane protein thiols (–SH)

Conditions	–SH (nmol/mg protein)
No Vimang addition	37.04 ± 3.56
10 μM Vimang	17.12 ± 3.48[a]
10 μM Vimang + 100 μM EGTA (added 5 min after Ca^{2+})	33.04 ± 4.22[b]
Control + 100 μM EGTA (added 5 min after Ca^{2+})	35.73 ± 2.49 ns
10 μM Vimang + 100 μM EGTA (initially added)	44.03 ± 1.29[a]
10 μM Vimang + 1 μM CsA	24.81 ± 1.46[a]
10 μM Vimang + 20 μM NEM	45.31 ± 2.03[a]

The experimental conditions are similar to those described in legend to Fig. 4. Isolated mitochondria (0.5 mg/ml) were added to the reaction medium described in the legend to Fig. 1. Mitochondrial membrane protein thiols content was determined as described in Section 2, after 10 min of incubation. The values represent average of three experiments ±S.D. using different mitochondrial preparations. ns: statistically not significantly different in relation to the control.
[a] $P < 0.05$, non-paired Student's test compared with condition a (control).
[b] $P < 0.05$, non-paired Student's test compared with condition b (Vimang treatment).

3.3. Effects of EGTA, cyclosporin A and NEM on Vimang-induced mitochondrial swelling and protein thiol oxidation

Fig. 4 shows that in presence of 20 μM Ca^{2+}, mitochondria underwent slight swelling, that was increased by the addition of 10 μM Vimang. The MPT inhibitors EGTA, cyclosporin A and NEM, strongly inhibited mitochondrial swelling elicited by Ca^{2+} plus Vimang. Table 2 shows the effects of the same MPT inhibitors on the oxidation of protein thiol groups by Ca^{2+} plus Vimang. A decrease of about 45% in the content of membrane protein thiols was observed; it could be prevented by EGTA and NEM, but not by CsA.

3.4. Effects of gallic acid, benzoic acid and catechin on MPT, and spectral changes of these compounds in the presence of H_2O_2/horseradish peroxidase

MPT-inducing ability was evaluated at 10 μM concentration of the above-cited representative components of Vimang, under the experimental conditions described in Fig. 2. All compounds showed some ability to induce CsA-sensitive swelling and therefore onset of MPT. The tracings obtained (not shown) were closely similar to those of Vimang/mangiferin; however, the extent of the effect of gallic acid, benzoic acid and catechin respectively, measured 10 min following the beginning

of swelling, were approximately, 10, 60 and 30% of that induced by Vimang. The spectrum of catechin (not shown), presented an absorption peak at around 430 nm, that increased over the time of incubation of the flavonoid with H_2O_2/horseradish peroxidase under the conditions previously described for mangiferin [12]; ascorbate abolished this effect while reduced glutathione completely eliminated the peak. The spectra of gallic and benzoic acids exposed to the same system, did not show important changes.

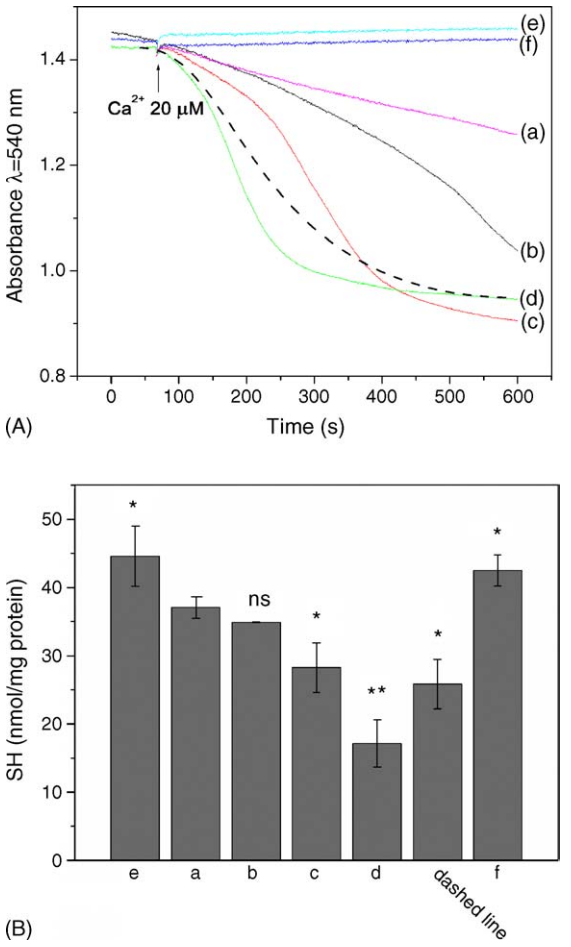

Fig. 2. Mitochondrial swelling (A) and oxidation of mitochondrial membrane protein thiols (B), elicited by Vimang/mangiferin. Mitochondria (0.5 mg/ml) were incubated as described in Section 2. The additions were: (a) no Vimang addition, (b) 1 μM Vimang, (c) 5 μM Vimang, (d) 10 μM Vimang, (e) 10 μM Vimang + 100 μM EGTA, (f) 10 μM Vimang + 1 μM RR (dashed line) 10 μM mangiferin. Ca^{2+} was added as indicated. Panel A shows a typical experiment; three additional experiments with different mitochondrial preparations yielded comparable results. In panel B results are expressed as means ± S.D. ($n = 3$). One tailed t-test was used for statistical analyses; $^{*}P < 0.05$, $^{**}P < 0.01$, n.s., not significant compared to condition (A).

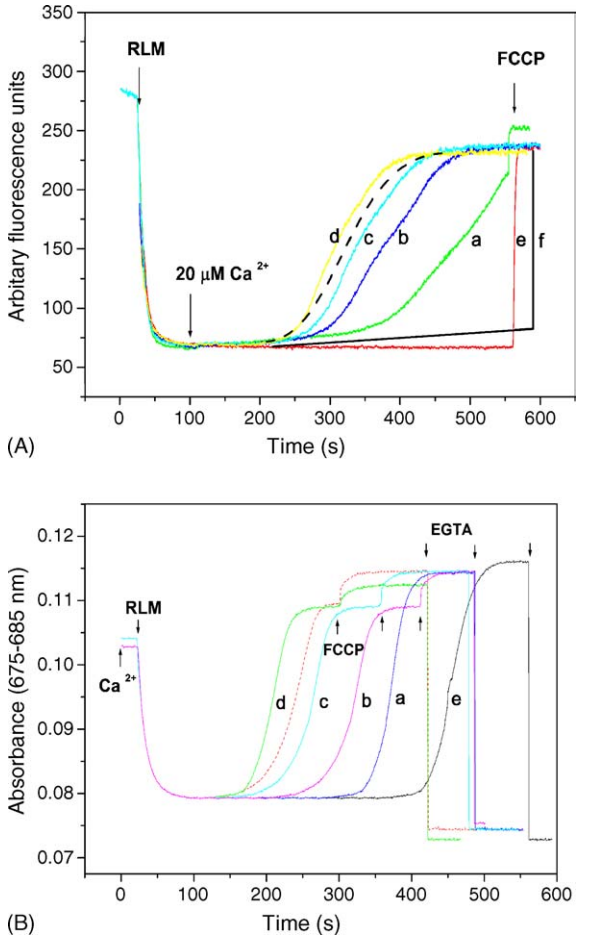

Fig. 3. Stimulation of Ca^{2+}-mediated loss of $\Delta\Psi$ (Panel A) and Ca^{2+} release (Panel B), elicited by Vimang/mangiferin. Experimental conditions are described in Section 2. The additions were: (a) no Vimang addition, (b) 1 μM Vimang, (c) 5 μM Vimang, (d) 10 μM Vimang, (e) 10 μM Vimang + 100 μM EGTA or + 1 μM CsA, (f) 10 μM Vimang + 20 μM NEM, dashed line 10 μM mangiferin. RLM (0.5 mg/ml), Ca^{2+} (20 μM), FCCP (1 μM, ascending arrows) and EGTA (100 μM, descending arrows) were added as indicated. Three additional experiments with different mitochondrial preparations, exhibited the same trend.

4. Discussion

In a previous study, we demonstrated that mangiferin, the main component of Vimang (16%), induced MPT and oxidation of mitochondrial protein thiols; a change in the spectrum of the compound when exposed to H_2O_2/horseradish peroxidase compatible with the formation of oxidation products, was also demonstrated [12]. In the present study, we demonstrate that the Vimang extract also led to MPT, as assessed by Ca^{2+}-dependent, CsA-sensitive mitochondrial swelling; under the same MPT conditions, the extract also led to oxida-

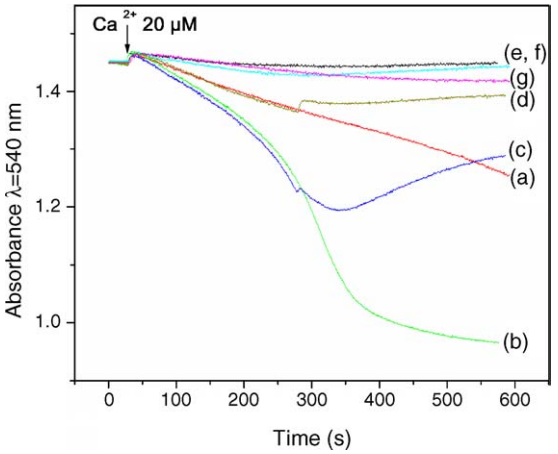

Fig. 4. Effects of EGTA, CsA and NEM on Vimang-elicited mitochondrial swelling. Mitochondria (0.5 mg/ml) were incubated in the reaction medium (2.0 ml, 28 °C) containing 125 mM sucrose, 65 mM KCl, 2 mM inorganic phosphate, 1 mM magnesium chloride, 10 mM HEPES buffer pH 7.2, a cocktail of NAD^+-linked substrates at 5 mM (malate, pyruvate, α-ketoglutarate) and (a) no Vimang addition, (b) 10 μM Vimang, (c) 10 μM Vimang + 100 μM EGTA (added 5 min after Ca^{2+}), (d) control + 100 μM EGTA (added 5 min after Ca^{2+}), (e) 10 μM Vimang + 100 μM EGTA (initially added), (f) 10 μM Vimang + 1 μM CsA, (g) 10 μM Vimang + 20 μM NEM. Ca^{2+} was added as indicated. The assays were performed three times using different mitochondrial preparations, with comparable results.

tion of mitochondrial protein thiol groups. The observed inhibition of Vimang-induced MPT and oxidation of protein thiols by EGTA and RR is believed to support strict Ca^{2+} involvement in this process. On the other hand, the finding that CsA inhibits the swelling, but not the oxidation of mitochondrial protein thiols, corroborates previous reports [19], supporting the concept that cyclophilin is a component needed for MPT onset stimulated by thiol oxidants [20]: prevention of cyclophilin binding by CsA inhibits MPT even when crucial thiols are oxidized. Similarly to mangiferin itself, and under the same experimental MPT conditions, Vimang also dissipated mitochondrial membrane's potential and released pre-accumulated Ca^{2+} from mitochondria, both important, well established consequences of MPT.

Along with the xanthone mangiferin, Vimang contains other polyphenols, including phenolic acids (gallic, 3,4-dihydroxy benzoic, and benzoic acids), phenolic esters (methyl gallate, propyl gallate, propyl benzoate) and flavan-3-ols (catechin/epicatechin). We evaluated the ability of MPT induction by the most representative of these compounds, e.g. gallic acid, benzoic acid and catechin. All showed some MPT-inducing ability, benzoic acid being most effective. This compound however, together with its derivatives, comprises only around 2% of the whole extract, i.e., considerably less than mangiferin's 16% content in Vimang; gallic acid, a poor MPT inducer, makes up around 5%. Catechin is the second main component of Vimang's extract comprising together with epicatechin, approximately 11% of it. Its MPT-inducing ability has been previously reported [21] and is reproduced here. It's swelling action on mitochondria however, was only approximately 30% of that elicited by mangiferin. Nevertheless, catechin's contribution to the overall effect of Vimang on MPT should be taken into account because like mangiferin, it is a catechol-containing polyphenol also displaying significant antioxidant activity (unpublished results). In this regard, a possible synergistic action of mangiferin and catechin/other polyphenols appears worth considering.

Although the above mentioned effects of Vimang (and mangiferin) is paradoxical for an extract having potent antioxidant activity [7,8,22], they could explain the recently reported ability of mangiferin to induce apoptosis in different cell lines [23,24]. Vimang is an effective antioxidant, apparently on account of its catechol-containing polyphenol contents, mangiferin and possibly catechin [11]. It has been proposed that, concomitantly with the protection afforded against free radicals, catechols undergo oxidation, giving rise to potentially toxic products containing semiquinone radicals and quinones, with ability to arylate protein thiol groups [5,6,25]. Indeed, a reference quinone, 4-methylorthobenzoquinone, reacts with protein thiols as efficiently as does the synthetic thiol-alkylating agent NEM [26]. Therefore, oxidation products of catechol-containing antioxidants present in Vimang, mangiferin in particular, show a potentiality to develop prooxidant activity via arylation of thiol groups [6]. In this regard, it is conceivable that exposure of mitochondria to Ca^{2+} stimulates ROS generation, whose reaction with mangiferin could produce quinoids derivatives capable of interacting with the more accessible mitochondrial protein thiols.

Prooxidant effects of polyphenols have been previously reported, including the activation to semiquinone/quinone derivatives by peroxidases [27,28]; the Ca^{2+} in our mitochondrial system might reproduce this effect. Semiquinone/quinine-type oxidation metabolites of catechol-containing polyphenols, may act as electrophiles, binding to cellular macromolecules or may result in the generation of ROS through redox cycling [27]. In this regard, the flavonoid quercetin contains a catechol group and may be oxidized to quinone/quinone methide intermediates, forming adducts with GSH [27–32]. In accordance with this view, in a previous study we evaluated the effect of the oxidizing H_2O_2/horseradish peroxidase system on the mangiferin

spectrum, obtaining results compatible with the formation of xanthone oxidation products, apparently able to form adducts with GSH [12]; in the present study, following a similar protocol for catechin as a Vimang compound, we observed a behavior differing from that of mangiferin, suggesting that another mechanism could be involved in MPT induction by that flavonoid. In the former study we had also demonstrated that treatment of rats with mangiferin, increased their mitochondria's susceptibility to MPT. In the same way, the compound presented antioxidant activity inhibiting Ca^{2+}-induced peroxidation of mitochondrial membrane lipids, as well as ROS generation by organelles [12].

Considering the above mentioned literature data, and including our own [12], we postulate that mangiferin reacts with Ca^{2+}-stimulated mitochondria-generated superoxide, giving rise to the o-semiquinone radical at the mangiferin catechol moiety, leading to o-quinone, due to its autoxidation or antioxidant action. Such electrophilic quinone type metabolites may be scavenged by GSH or others thiol-containing molecules not by means of chemical reduction, but rather by the formation of conjugates. In this regard, the formation of adducts between GSH and flavonoids has been recently demonstrated in a cellular in vitro system; it provides evidence for its relevance to biological systems and for the actual prooxidative formation of reactive quinone type metabolites from catechol-containing polyphenols [32]. Also, the toxicity of quercetin and structurally related polyphenols has been demonstrated by various mutagenicity tests, and is often associated with quinone/quinone methide chemistry [33]. Therefore, isomeric quinone methide derivatives of mangiferin are likely to present the potentiality of being responsible for the depletion of thiol groups observed in our mitochondrial system, which in turn is the most probable cause of MPT onset by Vimang.

It is worthy observing that Vimang/mangiferin effects on mitochondria are strictly dependent on Ca^{2+}, not significantly affecting the organelles by themselves. Quinones are known to decrease the respiratory control ratio (RCR) of mitochondria according to their oxidative (redox cycling) or electrophilic (arylating) reactivities [26,34]. Furthermore, some flavonoids are known to trigger a respiratory burst in isolated mitochondria undergoing auto-oxidation, thereby generating ROS [35]. Although Vimang/mangiferin caused RCR to decrease, the strict Ca^{2+}-dependence of this effect, as well as its CsA-sensitivity, are likely to associates it with MPT. On the other hand, this result would exclude redox cycling or direct ROS production as putative direct causes of the prooxidant effect of Vimang/mangiferin observed on mitochondria.

Mitochondria are the most important intracellular source of ROS, under either physiological or pathological conditions. It is our proposal that on normal mitochondria, mangiferin does not by itself, cause a prooxidant effect on organelles. However, under conditions of enhanced mitochondrial ROS generation, such as by exposure of the organelles to relative high Ca^{2+} levels, or when the antioxidant defense mechanism has been impaired, ROS accumulation could lead to increased levels of oxidized products of mangiferin, capable of interacting directly with mitochondrial membrane thiol groups, resulting in MPT onset and ultimately in cell damage. As mentioned above, catechin may have a participation in MPT onset by Vimang, although apparently by a mechanism differing from that of mangiferin.

Free radical scavengers afford protection against oxidative stress on account of their reaction with damaging reactive species. Regardless of this protection, the toxicity of the reactive oxidation products emerging on account of this antioxidant action might be substantial; this aspect should be considered when Vimang or other antioxidants [36] are employed as nutritional supplements. Nevertheless, while this effect is the opposite of the beneficial effect of Vimang's antioxidant activity, it could be beneficial for certain cancer cells exposed to an over-production of ROS. This condition, by triggering MPT-mediated apoptosis, may represent an important defense factor. It is conceivable that Vimang/mangiferin, by stimulating such a mechanism, could act as a prodrug, whose toxicity may be restricted to ROS-damaged cells.

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