Pharmacological Research, Vol. 42, No. 6, 2000
doi:10.1006/phrs.2000.0727, available online at http://www.idealibrary.com on IDEAL

PROTECTIVE EFFECTS OF *MANGIFERA INDICA* L. EXTRACT, MANGIFERIN AND SELECTED ANTIOXIDANTS AGAINST TPA-INDUCED BIOMOLECULES OXIDATION AND PERITONEAL MACROPHAGE ACTIVATION IN MICE

G. M. SÁNCHEZ[a,*], L. RE[b], A. GIULIANI[c], A. J. NÚÑEZ-SELLÉS[d], G. P. DAVISON[a] and O. S. LEÓN-FERNÁNDEZ[a]

[a]*Centre for Research and Biological Evaluation, Pharmacy Institute, Havana University, P.O. 10 400, Havana, Cuba,* [b]*Institute of Experimental and Clinical Medicine, Laboratory of Pharmacology, University of Ancona, Via Ranieri, 2, 60131 Ancona, Italy,* [c]*Department of Chemistry and Medical Biochemistry, University of Milan, Via Saldini, 50-20133 Milan, Italy,* [d]*Centre of Pharmaceutical Chemistry, P.O. Box. 16042, Havana, Cuba*

Accepted 11 July 2000

We compared the protective abilities of *Mangifera indica* L. stem bark extract (Vimang®) 50–250 mg kg^{-1}, mangiferin 50 mg kg^{-1}, vitamin C 100 mg kg^{-1}, vitamin E 100 mg kg^{-1} and β-carotene 50 mg kg^{-1} against the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative damage in serum, liver, brain as well as in the hyper-production of reactive oxygen species (ROS) by peritoneal macrophages. The treatment of mice with Vimang®, vitamin E and mangiferin reduced the TPA-induced production of ROS by the peritoneal macrophages by 70, 17 and 44%, respectively. Similarly, the H_2O_2 levels were reduced by 55–73, 37 and 40%, respectively, when compared to the control group. The TPA-induced sulfhydryl group loss in liver homogenates was attenuated by all the tested antioxidants. Vimang®, mangiferin, vitamin C plus E and β-carotene decreased TPA-induced DNA fragmentation by 46–52, 35, 42 and 17%, respectively, in hepatic tissues, and by 29–34, 22, 41 and 17%, in brain tissues. Similar results were observed in respect to lipid peroxidation in serum, in hepatic mitochondria and microsomes, and in brain homogenate supernatants. Vimang® exhibited a dose-dependent inhibition of TPA-induced biomolecule oxidation and of H_2O_2 production by peritoneal macrophages. Even if Vimang®, as well as other antioxidants, provided significant protection against TPA-induced oxidative damage, the former lead to better protection when compared with the other antioxidants at the used doses. Furthermore, the results indicated that Vimang® is bioavailable for some vital target organs, including liver and brain tissues, peritoneal exudate cells and serum. Therefore, we conclude that Vimang® could be useful to prevent the production of ROS and the oxidative tissue damages *in vivo*.

KEY WORDS: *Mangifera indica*, mangiferin, oxidative stress, vitamin C, vitamin E, β-carotene, free radicals.

INTRODUCTION

There is considerable interest in the role of reactive oxygen species (ROS) damage for the pathophysiology of some human diseases. ROS possess a strong oxidizing effect and induce damage to biological molecules, including proteins, lipids and DNA, with concomitant changes in their structure and function [1]. Increased quantities of the oxidized metabolites of these molecules have been detected in patients with different clinical conditions [2]. Humans, such as other aerobic organisms, have developed several protective mechanisms against the deleterious effects of ROS. However, the extensive generation of ROS in some pathological conditions appears to overwhelm natural defense mechanisms, thereby reducing dramatically the levels of endogenous antioxidants [3]. If a human disease can be attributed to an imbalance of its antioxidant defenses due to oxidative stress then, theoretically, it should be possible to limit the oxidative damage and prevent the progression of the disease by reinforcing the antioxidant defenses [2, 4]. Epidemiological studies support the hypothesis that the major antioxidant nutrients vitamin E, vitamin C and β-carotene may play a beneficial role in the prevention of several chronic disorders [5]. The therapeutic potentials

*Corresponding author. E-mail: gregorio@infomed.sld.cu

1043–6618/00/120565–09/$35.00/0

of vegetable constituents have aroused a certain interest in many laboratories [2, 6]. The problems that arise are related mainly to the study of the bioactivity of these compounds from a strict pharmacological point of view [5].

Vimang®, an extract obtained from the stem bark of selected varieties of *Mangifera indica* L., contains a defined mixture of components (polyphenols, terpenoids, steroids, fatty acids and microelements) [7] and shows a very potent scavenger activity for hydroxyl radicals (OH·) and hypochlorous acid, a significant inhibitory effect on the peroxidation of rat brain phospholipids, and a protective effect against DNA damage induced by iron/bleomycin or copper-phenantroline models [8]. Vimang® has been tested in a broad set of toxicological tests with satisfactory results, including acute and subcronic toxicity, genotoxicity, and irritability and is classified as a nontoxic product. Some of the main components of Vimang® (i.e. mangiferin, amentoflavone, friedelin, daucosterol and beta-sitosterol) have been reported to show antioxidant properties in some *in vitro* models [9–11]. This work deals with the protective antioxidant abilities of a *Mangifera indica* L. bark extract (Vimang®) and mangiferin, the main polyphenol of Vimang® (20%), compared to the vitamin C, vitamin E acetate and β-carotene effects *in vivo*.

MATERIALS AND METHODS

Drugs

Stem bark extract of *Mangifera indica* L. was prepared by decoction with a polar solvent for 1 hour. The extract was concentrated by evaporation and spray dried to obtain a fine brown powder, coded as QF808, which was used as the 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 [7]. The solid extract was dissolved in distilled water for pharmacological studies.

Mangiferin (2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-one) was supplied by the Centre of Pharmaceutical Chemistry (Cuba). It was purified from QF 808 by extraction with methanol and its purity (90%) was assessed.

Animals and treatment

Male OF1 mice (20–30 g) were obtained from CENPALAB (Bejucal, Havana, Cuba). The animals were housed in a controlled environment at 20 ± 2 °C (12 h light and 12 h dark cycle) and acclimatized 7 days before experiments. All animals were allowed to have free access to food (Standard diet for rodent, CENPALAB) and tap water. Vitamin E and β-carotene were dissolved in sunflower oil, whereas Vimang®, mangiferin and vitamin C were dissolved in water. Vimang® (50–250 mg kg^{-1}), mangiferin (50 mg kg^{-1}), vitamin C (100 mg kg^{-1}), vitamin E (100 mg kg^{-1}) and β-carotene (50 mg kg^{-1}) were orally administered once a day to groups of animals with the use of a feeding needle for 7 consecutive days. All the treatments were carried out daily in the morning between 8:30 A.M. and 9:30 A.M. All mice groups received an intraperitoneal (IP) injection of 1 ml of 3% thioglycolate broth 3 days before TPA treatment to elicit peritoneal macrophages [12]. TPA was administered on the eighth day 2 hours after the antioxidant treatment. Groups of mice were individually treated IP with 0.1 μg TPA diluted in 1 ml of sterile phosphate-buffered saline (PBS) solution to induce an oxidative stress. The control group received PBS buffer and were anesthetized (pentobarbital 50 mg kg^{-1}, intramuscular) 2 hours post treatment. The peritoneal macrophage cells were isolated, blood samples taken from abdominal aorta, the hepatic and brain tissues quickly removed, and the subcellular fractions obtained as described elsewhere [13]. All procedures were performed as approved by the institutional Animals Care Committees and in accordance with the European Union Guidelines for Animals Experimentation.

Biochemical determinations

Biochemical parameters were determined by spectrophotometric methods using an Ultrospect III Plus Spectrophotometer (Pharmacia-LKB, Sweden). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured in total blood samples using a kit supplied by Randox Laboratories Ltd., Ireland (Cat. No. SD125 and No. RS505). The total sulfhydryl group protein content (*TSH*) was assessed according to the method of Sedlak and Lindsay (1968) with Ellman's reagent [14]. Malondialdehyde (MDA) plus 4-hydroxyalkenals (4-HA) were assayed by a colorimetric method (586 nm) (Bioxytech LPO-586 kit, Oxis International, Portland, OR, USA) as a marker of lipid peroxidation (LP) utilizing 1-methyl-2-phenylindol as the chromogenic reagent [15]. For the determination of peroxidation potential (PP), serum was incubated with copper sulfate (2 mM, final concentration) at 37 °C for 24 hours. PP was estimated by taking the difference between LP values (MDA + 4-HA) at 24 and 0 hours [16]. The proteins were measured by a standard Coomassie Blue method [17]. Quantification of H_2O_2in peritoneal macrophages was measured by the Bioxytech H_2O_2-560 kit (Oxis International Inc., USA) using xilenol orange to form a stable coloured complex, which was measured at 560 nm. The reaction mixture contained 1 ml of reagent and 50 μl of macrophages (3×10^6 cells ml^{-1}). Values were expressed as μM/3×10^6 cells.

Superoxide anion production by peritoneal macrophages was measured by cytochrome c reduction assay [18]. The reaction mixture, which was incubated for 15 min at 37 °C contained 1 ml of macrophages (3×10^6 cells ml^{-1}) and 0.05 mM cytochrome c. Placing the reaction mixture in ice terminated the reaction. The mixture

was centrifuged at 1500 ***g*** for 10 min at 4 °C, and the supernatant fraction was transferred to clean tubes for subsequent spectrophotometric measurement at 550 nm. Absorbance values were converted into nanomoles of reduced cytochrome c by using the extinction coefficient of 2.1×10^4 M cm^{-1} every 15 minutes [13]. For DNA fragmentation assays liver and brain samples were homogenized in a lisis buffer (5 mM Tris-HCl, 20 mM EDTA, 0.5% Triton X-100, pH 8.0). Homogenates were centrifuged at 27 000 ***g*** for 20 min to separate intact chromatin in the pellets from fragmented DNA in the supernatant fractions. Pellets were resuspended in 0.5 N perchloric acid, and 5.5 N perchloric acid was added to supernatant samples to reach a concentration of 0.5 N. Samples were heated at 90 °C for 15 min and centrifuged at 1500 ***g*** for 10 min to remove protein. Resulting supernatant was placed to react with Burton's reagent (diphenylamine 1.5 g in 100 ml of acetic acid and 1.5 ml of H_2SO_4 with acetaldehyde at the final concentration of 16 μg ml^{-1}) for 20 h at room temperature and adsorbance was measured at 600 nm [19]. DNA fragmentation was expressed as a percentage of total DNA appearing in the supernatant fraction [20].

Statistical analysis

The OUTLIERS preliminary test for the detection of error values was initially applied as statistical analysis. Afterward, ANOVA (single way) was used followed by a homogeneity variance test (Bartlett–Box). In addition, a multiple comparison test was used (Duncan test). Data were expressed as the mean ± standard deviation of five animals. The level of statistical significance employed was at least $P < 0.05$ for all the experiments.

RESULTS

Antioxidant enzymes in blood samples

Table I reports the effects of treatments on SOD and GPx blood levels. As compared to the control group, combination of vitamin E plus vitamin C (100 mg kg^{-1} each), β-carotene (50 mg kg^{-1}), mangiferin (50 mg kg^{-1}) and Vimang® (250 mg kg^{-1}) increased approximately 1.4-fold the SOD levels. TPA induced an increase in SOD serum level for all groups, with the higher levels in β-carotene-, Vimang®- and mangiferin-treated groups as compared to the control.

No significant modification in GPx levels was observed after the treatment of mice with all the tested antioxidants. TPA administration decreased the GPx level by 2.1-fold, as compared to the control group. Only the Vimang® treated animals (250 mg kg^{-1}) preserved GPx at its normal level. Mangiferin (50 mg kg^{-1}) showed no effect on GPx levels depleted by TPA, however the other antioxidants attenuated the depletion of GPx induced by TPA.

Production of ROS by peritoneal macrophages

The results related to the cytochrome c reduction and to the H_2O_2 production in peritoneal exudate cells (primarily macrophages) are shown in Table II. The effect of TPA on the production of superoxide anion by peritoneal macrophages indicated that neither Vimang®, mangiferin, vitamin C nor β-carotene modified the superoxide anion production in the absence of TPA. A 3.3-fold increase in superoxide anion production was observed in the peritoneal macrophages treated with vitamin E alone in accordance with previous reports [21]. This effect takes place due to the dual role of vitamin E, which can induce either an antioxidant or a pro-oxidant action.

On the basis of cytochrome c reduction as compared to the cells from untreated animals, TPA administration induced a 9.7-fold increase in production of the superoxide anion (see Table II). The administration of Vimang® 50, 110 and 250 mg kg^{-1} for 7 consecutive days decreased TPA-induced cytochrome c reduction by approximately 70%. Pretreatment of animals with vitamin C (100 mg kg^{-1}), vitamin E (100 mg kg^{-1}) and a combination of vitamin C and vitamin E (100 mg kg^{-1} each) decreased the TPA-induced cytochrome c reduction by 6, 17, and 44%, respectively, as compared to the control samples. Administration of mangiferin (50 mg kg^{-1}) and β-carotene (50 mg kg^{-1}) for 7 consecutive days decreased the TPA-induced cytochrome c reduction by 44 and 16%, respectively.

The concentration of H_2O_2 produced by peritoneal macrophages from the TPA-treated animals (see Table II), was increased 2.7-fold when compared to the control group. Any significant increase in H_2O_2 levels was observed after the treatment of the mice either with Vimang®, vitamin C or β-carotene. A 1.7-fold increase in H_2O_2 concentration was observed in the peritoneal macrophages of the animals treated with vitamin E.

Vimang® inhibited in a dose-dependent fashion the H_2O_2 production induced by TPA. The administration of Vimang® 50, 110 and 250 mg kg^{-1} for 7 consecutive days decreased the TPA-induction of H_2O_2 levels by 55, 63 and 73%, respectively, as compared to the control values. Pretreatment with vitamin C (100 mg kg^{-1}), vitamin E (100 mg kg^{-1}), vitamin C plus vitamin E (100 mg kg^{-1} each) and Vimang® (110 mg kg^{-1}) decreased the TPA-induced H_2O_2 production by 19, 37, 24 and 63%, respectively, as compared to the control samples. The administration of β-carotene (50 mg kg^{-1}), mangiferin (50 mg kg^{-1}) and Vimang® (50 mg kg^{-1}) for 7 consecutive days decreased the TPA-induced H_2O_2 production by 15, 40 and 55%, respectively.

Sulfhydryl oxidation

No significant changes were found in *TSH* levels, mainly glutathion, in supernatant of brain homogenates or serum samples of the animals used in this experiment (Table III). After the treatment with TPA, a decrease in *TSH* by 1.6-fold was observed in supernatant of hepatic

Table I
Effects of TPA on SOD and GPx levels in mice blood samples and comparative effects of Vimang®, mangiferin and selected antioxidants

	SOD $U\ g^{-1}$ *haemoglobin*	*GPx* $U\ g^{-1}$ *haemoglobin*
Control	37.5 ± 5.4^{a}	149.0 ± 21.8^{a}
Sunflower oil	38.5 ± 2.6^{a}	150.9 ± 18.3^{a}
Vitamin C (100 mg kg^{-1})	43.0 ± 6.5^{a}	167.3 ± 19.4^{a}
Vitamin E (100 mg kg^{-1})	39.4 ± 1.3^{a}	148.9 ± 22.7^{a}
Vitamin C and E (100 mg kg^{-1} each)	56.3 ± 6.0^{b}	147.6 ± 19.6^{a}
β-carotene (50 mg kg^{-1})	55.7 ± 4.7^{b}	108.8 ± 15.5^{a}
Vimang® (250 mg kg^{-1})	53.4 ± 5.7^{b}	114.5 ± 18.8^{a}
Mangiferin (50 mg kg^{-1})	56.5 ± 4.8^{b}	117.4 ± 17.2^{a}
TPA	52.7 ± 8.7^{b}	69.1 ± 14.1^{b}
TPA + vitamin C (100 mg kg^{-1})	44.4 ± 2.1^{ab}	109.5 ± 16.2^{c}
TPA + vitamin E (100 mg kg^{-1})	45.2 ± 8.9^{ab}	84.6 ± 10.1^{bc}
TPA + vitamin C and E (100 mg kg^{-1} each)	55.2 ± 3.8^{b}	98.3 ± 19.8^{bc}
TPA + β-carotene (50 mg kg^{-1})	52.3 ± 7.5^{bc}	88.2 ± 13.7^{bc}
TPA + Vimang® (50 mg kg^{-1})	65.4 ± 3.2^{c}	99.6 ± 18.3^{bc}
TPA + Vimang® (110 mg kg^{-1})	61.9 ± 7.1^{bc}	107.2 ± 10.3^{c}
TPA + Vimang® (250 mg kg^{-1})	65.0 ± 5.1^{c}	131.3 ± 23.8^{ac}
TPA + mangiferin (50 mg kg^{-1})	63.9 ± 2.9^{c}	59.3 ± 19.8^{b}

Male OF1 mice were treated with a single dose of TPA (0.1 μg) after receiving antioxidant(s) for 7 days. SOD and GPx levels were assayed in blood samples using a standard Randox diagnostic kit. Each value represents the mean ± SD of five mice. Values with non-identical superscripts are significantly different ($P < 0.05$) within the same set.

Table II
Production of reactive oxygen species by peritoneal macrophages based on cytochrome c reduction and H_2O_2 levels after treatment of mice with TPA, and the comparative scavenging abilities of Vimang®, mangiferin and selected antioxidants

	Cytochrome c reduction *nmol every 15 min per* 3×10^6 *cells*	H_2O_2 *levels* μM *per* 3×10^6 *cells*
Control	2.39 ± 0.93^{a}	929 ± 94^{a}
Sunflower oil	3.29 ± 0.73^{a}	880 ± 92^{a}
Vitamin C (100 mg kg^{-1})	2.42 ± 0.84^{a}	825 ± 70^{a}
Vitamin E (100 mg kg^{-1})	7.86 ± 0.59^{b}	1618 ± 116^{b}
Vitamin C and E (100 mg kg^{-1} each)	7.58 ± 0.55^{b}	1495 ± 154^{b}
β-carotene (50 mg kg^{-1})	3.83 ± 0.84^{a}	877 ± 105^{a}
Vimang® (250 mg kg^{-1})	3.48 ± 0.25^{a}	763 ± 135^{a}
Mangiferin (50 mg kg^{-1})	3.41 ± 0.77^{a}	850 ± 111^{a}
TPA	23.19 ± 1.31^{c}	2517 ± 367^{c}
TPA + vitamin C (100 mg kg^{-1})	21.74 ± 1.48^{c}	2043 ± 352^{c}
TPA + vitamin E (100 mg kg^{-1})	19.16 ± 1.39^{d}	1574 ± 282^{b}
TPA + vitamin C and E (100 mg kg^{-1} each)	13.03 ± 1.40^{e}	1912 ± 245^{c}
TPA + β-carotene (50 mg kg^{-1})	19.43 ± 1.68^{d}	2143 ± 195^{ab}
TPA + Vimang® (50 mg kg^{-1})	$6.16 \pm 1.25^{b*}$	1143 ± 168^{a}
TPA + Vimang® (110 mg kg^{-1})	$6.96 \pm 1.59^{b*}$	$938 \pm 103^{a*}$
TPA + Vimang® (250 mg kg^{-1})	$7.62 \pm 1.00^{b*}$	$688 \pm 198^{a*}$
TPA + mangiferin (50 mg kg^{-1})	13.02 ± 1.09^{e}	1495 ± 215^{b}

Male OF1 mice were treated with a single dose of TPA (0.1 μg) after receiving antioxidant(s) for 7 days. Peritoneal exudate cells (primarily macrophages) were analysed for cytochrome c reduction and H_2O_2 levels. Each value represents the mean ± SD of five mice. Values with non-identical superscripts are significantly different ($P < 0.05$) within the same set. The * indicates a significance level of $P < 0.01$.

homogenates, as compared to the control values. The administration of Vimang® 250, 110 and 50 mg kg^{-1} for 7 days decreased the TPA-induced hepatic sulfhydryl loss by 50, 48, and 39%, respectively, as compared to the control values. The treatment with vitamin C (100 mg kg^{-1}), vitamin E (100 mg kg^{-1}), with a combination of both the C and E vitamins (100 mg kg^{-1} each) and Vimang® (110 mg kg^{-1}) for 7 days decreased the TPA-induced sulfhydryl oxidation by 15, 32, 42 and 48%, respectively, as compared to the control values. Pretreatment of mice with β-carotene (50 mg kg^{-1}), mangiferin (50 mg kg^{-1}) or Vimang® (50 mg kg^{-1})

Table III
Effects of TPA on sulfhydryl groups in mice serum, brain, and liver homogenates, and the comparative protective abilities of Vimang®, mangiferin and selected antioxidants

		Homogenates	
	Serum μM *of SH*	*Liver* nmol *of SH* mg^{-1}	*Brain protein*
Control	490.1 ± 50.0	2.22 ± 0.13^{a}	0.31 ± 0.03
Sunflower oil	415.5 ± 77.0	2.02 ± 0.13^{a}	0.28 ± 0.05
Vitamin C (100 mg kg^{-1})	385.4 ± 48.2	1.85 ± 0.30^{a}	0.32 ± 0.04
Vitamin E (100 mg kg^{-1})	377.9 ± 68.7	2.10 ± 0.15^{a}	0.30 ± 0.04
Vitamin C and E (100 mg kg^{-1} each)	412.2 ± 56.9	2.40 ± 0.13^{a}	0.27 ± 0.04
β-carotene (50 mg kg^{-1})	496.6 ± 49.0	2.44 ± 0.27^{a}	0.31 ± 0.03
Vimang® (250 mg kg^{-1})	478.2 ± 47.8	2.23 ± 0.23^{a}	0.32 ± 0.05
Mangiferin (50 mg kg^{-1})	511.1 ± 36.2	2.28 ± 0.21^{a}	0.33 ± 0.04
TPA	487.4 ± 51.9	1.41 ± 0.16^{b}	0.34 ± 0.03
TPA + vitamin C (100 mg kg^{-1})	460.7 ± 38.9	1.62 ± 0.25^{bc}	0.33 ± 0.03
TPA + vitamin E (100 mg kg^{-1})	505.8 ± 54.3	1.87 ± 0.14^{c}	0.32 ± 0.03
TPA + vitamin C and E (100 mg kg^{-1} each)	541.8 ± 63.3	2.01 ± 0.23^{ac}	0.34 ± 0.02
TPA + β-carotene (50 mg kg^{-1})	494.9 ± 37.7	1.72 ± 0.13^{c}	0.33 ± 0.01
TPA + Vimang® (50 mg kg^{-1})	502.5 ± 35.6	1.97 ± 0.12^{ac}	0.34 ± 0.03
TPA + Vimang® (110 mg kg^{-1})	514.2 ± 21.9	2.10 ± 0.22^{ac}	0.34 ± 0.02
TPA + Vimang® (250 mg kg^{-1})	535.9 ± 37.0	2.13 ± 0.22^{ac}	0.35 ± 0.02
TPA + mangiferin (50 mg kg^{-1})	550.1 ± 53.3	2.17 ± 0.17^{ac}	0.34 ± 0.02

Male OF1 mice were treated with a single dose of TPA (0.1 μg) after receiving antioxidant(s) for 7 days. NPSH determinations were performed with Ellman's reagent. Each value represents the mean $\pm$ SD of five mice. Values with non-identical superscripts are significantly different ($P < 0.05$).

for 7 days reduced TPA-induced sulfhydryl loss by 21, 53 and 50%, respectively, relative to the respective control values.

DNA fragmentation

The TPA-induced DNA fragmentation in hepatic and brain tissues and the comparative protective abilities of various antioxidants are summarized in Table IV. TPA induced a 2.3-fold and a 1.7-fold increase in DNA fragmentation in the hepatic and brain tissues of mice, respectively, as compared to the controls. Any significant increase in DNA fragmentation was observed either with Vimang®, mangiferin, vitamin C or β-carotene. A 1.4-fold increase in DNA fragmentation was observed in the liver tissue, as compared to the control animals after the treatment with vitamin E alone, similarly to the previous report of Bagchi *et al.* [21]. Furthermore, no differences were found in the brain tissue with the same treatment.

Vimang® demonstrated a dose-dependent protective ability against the TPA-induced DNA fragmentation. The administration of 50, 110 and 250 mg kg^{-1} to the animals for 7 days decreased the TPA-induced hepatic DNA fragmentation by 46, 48, and 52%, respectively, as compared to control group, and DNA fragmentation decreased by approximately 29, 30 and 34% in the brain tissues at the same concentrations. The treatment with mangiferin (50 mg kg^{-1}) reduced by 35 and 22% the DNA fragmentation induced by TPA in liver and brain tissues, respectively. A pretreatment of the animals with vitamin C (100 mg kg^{-1}), vitamin E (100 mg kg^{-1}), a combination of vitamin C plus vitamin E (100 mg kg^{-1} each) and Vimang® (110 mg kg^{-1}) decreased the TPA-induced hepatic DNA fragmentation by 0, 27, 42 and 48%, respectively, under the same conditions. DNA fragmentation was reduced by 29, 23, 41 and 30% in brain tissues, respectively, as compared to the control samples. The administration of β-carotene (50 mg kg^{-1}) for 7 days reduced TPA-induced hepatic and brain DNA fragmentation by 17%, relative to the respective control values.

Lipid peroxidation

The effect of TPA and antioxidants on LP in hepatic mitochondria and microsomes, in serum and in supernatant of brain homogenates are summarized in Table V. Any significant effect on LP was observed with Vimang®, mangiferin, vitamin C or β-carotene. A 1.4-fold and 1.3-fold increase in LP were observed in the hepatic microsomes and brain homogenates, respectively, as compared to control animals after treatment with vitamin E. More, an increase by 1.6-fold was noted when combining vitamins C and E. However, following the treatment of mice with TPA, LP was increased by 2.0-, 2.0-, 2.8- and 1.3-fold, respectively in the hepatic mitochondria and microsomes, in supernatant of brain homogenates and in serum. A 7-day pretreatment of the animals with 50, 110 and 250 mg kg^{-1} of Vimang® reduced the TPA-induced hepatic mitochondria LP by 22, 24 and 34%, respectively. In hepatic microsomal fractions, the observed reduction was of 17, 23 and 33%, respectively. Moreover, the administration of Vimang® at the same concentrations protected

Table V
TPA-induced lipid peroxidation in mice serum, hepatic mitochondria and microsomes, and in brain homogenates, and the comparative protective abilities of Vimang® and selected antioxidants

	Serum		*Liver (MDA + 4-HA)*		
	PP μM	*MDA + 4-HA* μM	*Microsomes*	*Mitochondria* $\mu mol\ mg^{-1}$ *protein*	*Brain MDA + 4-HA*
Control	14.6 ± 1.9^{a}	11.7 ± 1.9^{a}	4.23 ± 0.75^{a}	3.14 ± 0.32^{a}	1.69 ± 0.16^{a}
Sunflower oil	12.9 ± 1.6^{a}	10.1 ± 0.9^{a}	4.26 ± 0.70^{a}	3.11 ± 0.46^{a}	1.77 ± 0.19^{a}
Vitamin C (100 mg kg^{-1})	13.6 ± 1.7^{a}	10.2 ± 1.3^{a}	4.38 ± 0.63^{a}	2.78 ± 0.38^{a}	1.81 ± 0.19^{a}
Vitamin E (100 mg kg^{-1})	11.7 ± 1.2^{b}	10.6 ± 1.0^{a}	6.07 ± 0.69^{b}	3.53 ± 0.29^{ad}	2.23 ± 0.26^{b}
Vitamin C and E(100 mg kg^{-1} each)	11.7 ± 1.0^{b}	10.4 ± 0.7^{a}	6.82 ± 0.56^{b}	2.92 ± 0.44^{a}	2.32 ± 0.27^{b}
β-carotene(50 mg kg^{-1})	14.0 ± 1.8^{a}	10.9 ± 0.6^{a}	4.96 ± 0.47^{a}	3.13 ± 0.30^{a}	1.92 ± 0.24^{ab}
Vimang® (250 mg kg^{-1})	12.0 ± 1.0^{ab}	10.9 ± 1.0^{a}	4.66 ± 0.48^{a}	3.17 ± 0.36^{a}	1.67 ± 0.38^{ab}
Mangiferin (50 mg kg^{-1})	14.2 ± 1.2^{a}	11.1 ± 1.6^{a}	4.79 ± 0.61^{a}	2.86 ± 0.48^{c}	2.34 ± 0.20^{b}
TPA	19.5 ± 0.5^{c}	15.8 ± 1.6^{b}	8.58 ± 0.93^{c}	6.16 ± 0.32^{b}	4.74 ± 0.38^{c}
TPA + vitamin C (100 mg kg^{-1})	15.7 ± 1.6^{a}	15.6 ± 1.5^{b}	9.16 ± 0.41^{c}	5.91 ± 0.33^{c}	4.40 ± 0.42^{c}
TPA + vitamin E (100 mg kg^{-1})	12.4 ± 1.6^{ab}	11.6 ± 0.6^{a}	5.80 ± 0.91^{ab}	4.03 ± 0.36^{d}	2.50 ± 0.34^{b}
TPA + vitamin C and E (100 mg kg^{-1} each)	11.7 ± 0.6^{b}	11.1 ± 0.8^{a}	6.00 ± 0.65^{b}	5.22 ± 0.25^{e}	2.08 ± 0.28^{b}
TPA + β-carotene (50 mg kg^{-1})	15.3 ± 1.3^{a}	11.9 ± 0.8^{a}	7.29 ± 0.86^{bc}	5.70 ± 0.32^{bce}	4.07 ± 0.45^{c}
TPA + Vimang® (50 mg kg^{-1})	11.1 ± 1.9^{ab}	11.9 ± 1.0^{a}	7.10 ± 0.70^{bc}	4.83 ± 0.41^{e}	2.57 ± 0.13^{b}
TPA + Vimang® (110 mg kg^{-1})	11.1 ± 1.4^{b}	11.7 ± 0.4^{a}	6.59 ± 0.86^{b}	4.70 ± 0.27^{f}	2.41 ± 0.21^{b}
TPA + Vimang® (250 mg kg^{-1})	11.2 ± 0.9^{b}	11.6 ± 1.2^{a}	5.77 ± 0.19^{b}	4.08 ± 0.44^{df}	1.95 ± 0.32^{ab}
TPA + mangiferin (50 mg kg^{-1})	13.3 ± 1.0^{ab}	11.8 ± 0.7^{a}	7.42 ± 0.75^{bc}	5.24 ± 0.32^{ef}	2.88 ± 0.32^{b}

Male OF1 mice were treated with a single dose of TPA (0.1 μg) after receiving antioxidant(s) for 7 days. Malondialdehyde (MDA) plus 4-hydroxyalkenals (4-HA) were assayed using a colorimetric reaction with 1-methyl-2-phenylindole as cromogen. PP, was estimated by taking the difference between LP value (MDA + 4-HA) at 24 and 0 hours, incubated with copper. Each value represents the mean ± SD of five mice. Values with non-identical superscripts are significantly different ($P < 0.05$) within the same set.

against the TPA induced LP in brain homogenates. Decreases in LP and PP by 26 and 43%, respectively, were found in serum at the same concentration, but without any apparent dose-effect relationship among the assayed concentrations. LP in serum of the group treated with all the Vimang® doses, in the presence of TPA, reached similar levels in respect to the control group without TPA. Moreover, the levels of PP in all the tested Vimang® concentrations, with or without TPA, were lower than the normal level in the PP saline control group.

Administration of vitamin C (100 mg kg^{-1}), vitamin E (100 mg kg^{-1}), a combination of vitamin C and vitamin E (100 mg kg^{-1} each) and Vimang® (110 mg kg^{-1}) for 7 days decreased TPA-induced LP in hepatic mitochondria by 4, 35, 15 and 24%, respectively. Similarly, the reduction in hepatic microsomes was 0, 21, 30 and 23%; in brain homogenates it was 7, 47, 56 and 49%, and in serum it was 27, 30, 24 and 26%, respectively. Furthermore, after the treatment with the same antioxidants the values of PP against TPA-induced LP in serum were decreased by 19, 36, 40 and 43%, respectively,.

Administration of β-carotene (50 mg kg^{-1}) decreased TPA-induced LP in hepatic mitochondria and microsomes, brain homogenates, and serum by 7, 15, 14 and 24%, respectively. Under the same conditions, a reduction by 21% in PP was observed in serum. Administration of mangiferin 50 mg kg^{-1} reduced TPA-induced LP by 15, 13, 39 and 25% in hepatic mitochondria and microsomes, brain homogenates and serum, respectively, as compared to the control values. In the same way, a reduction in PP by 15% was observed in the mangiferin plus TPA-induced LP group. In all the cases the reduction of LP with mangiferin 50 mg kg^{-1} was lower when compared to the values reached with the Vimang® 250 mg kg^{-1} plus TPA-induced LP group.

DISCUSSION

A diet rich in fresh fruit and vegetables is protective against cardiovascular diseases and other oxidative stress-induced disorders, including cancer [22]. Such chemoprotective properties have been attributed, in large part, to the antioxidant content, including vitamin C, vitamin E, β-carotene, and mineral micronutrients. Furthermore, plant-derived phenols may also exert a significant protective role. The biological, pharmacological and medical properties of polyphenolics have been extensively reviewed [23, 24]. Plant-derived phenols are reported, in addition to their free-radical scavenging and antioxidant activity, to have a broad spectrum of biological activities. Mangiferin (the main polyphenol of Vimang®) has been tested *in vitro* for its antioxidant [10, 25, 26], immuno-stimulating and antiviral properties [27]. Aglycone (norathyriol) was also tested *in vitro* as inhibitor of the formylmethionyl-leucyl-phenylalanine induced respiratory burst in rat neutrophils [11]. The second main polyphenol in Vimang® extract (10%), amentoflavone (biflavone), has been also identified as a COX inhibitor [28], as

Table IV
TPA-induced DNA fragmentation in mice hepatic and brain tissues, and the comparative protective abilities of Vimang® and selected antioxidants

	Homogenates	
	Liver (%)	*Brain (%)*
Control	2.19 ± 0.37^{a}	2.45 ± 0.31^{a}
Sunflower oil	2.17 ± 0.42^{a}	2.65 ± 0.14^{a}
Vitamin C (100 mg kg^{-1})	2.69 ± 0.45^{ab}	2.78 ± 0.32^{a}
Vitamin E (100 mg kg^{-1})	3.16 ± 0.27^{b}	2.72 ± 0.41^{a}
Vitamin C and E (100 mg kg^{-1} each)	3.53 ± 0.22^{b}	2.29 ± 0.20^{a}
β-carotene (50 mg kg^{-1})	2.23 ± 0.22^{a}	1.89 ± 0.49^{a}
Vimang® (250 mg kg^{-1})	2.44 ± 0.37^{a}	2.39 ± 0.28^{a}
Mangiferin (50 mg kg^{-1})	2.51 ± 0.22^{a}	2.90 ± 0.15^{a}
TPA	4.96 ± 0.34^{c}	4.17 ± 0.27^{b}
TPA + vitamin C (100 mg kg^{-1})	4.97 ± 0.40^{c}	2.97 ± 0.19^{c}
TPA + vitamin E (100 mg kg^{-1})	3.62 ± 0.44^{b}	3.22 ± 0.17^{c}
TPA + vitamin C and E (100 g kg^{-1} each)	2.88 ± 0.39^{ab}	2.46 ± 0.19^{a}
TPA + β-carotene (50 mg kg^{-1})	4.13 ± 0.28^{c}	3.46 ± 0.37^{c}
TPA + Vimang® (50 mg kg^{-1})	2.69 ± 0.26^{ab}	2.96 ± 0.29^{ac}
TPA + Vimang® (110 mg kg^{-1})	2.59 ± 0.12^{a}	2.94 ± 0.32^{ac}
TPA + Vimang® (250 mg kg^{-1})	2.40 ± 0.23^{a}	2.74 ± 0.28^{ac}
TPA + mangiferin (50 mg kg^{-1})	3.23 ± 0.44^{b}	3.24 ± 0.48^{c}

Male OF1 mice were treated with a single dose of TPA (0.1 μg) after receiving antioxidant(s) for 7 days. DNA fragmentation was measured spectrophotometrically by using Burton's reagent. Each value represents the mean ± SD of five mice. Values with non-identical superscripts are significantly different ($P < 0.05$) within the same set.

an inhibitor of microsomal lipid peroxidation [9], and as an inhibitor of degranulation and arachidonic acid release in rat neutrophils [29]. The presence of selenium as an organic compound in Vimang® (0.05%) is also important for the *in vivo* antioxidant mechanism of the same extract.

For a compound to be defined as an antioxidant, it must satisfy two basic conditions: (1) to be present in low concentrations relative to the substrate to be oxidized and for its ability to delay or prevent auto-oxidation or free radical-mediated oxidative injury and (2) the resulting product formed after scavenging must be stable through intramolecular hydrogen bonding on further oxidation [30]. Therefore, *in vivo* assays are needed to state the bioactivity and bioavailability of this polyphenolic extract.

In the present study, the protective abilities of Vimang®, a *Mangifera indica* L. bark extract, mangiferin, vitamin C, vitamin E, a combination of vitamin C plus vitamin E and β-carotene were assessed on TPA-induced oxidative damage in serum, in hepatic and brain tissues, as well as in the activation of peritoneal macrophages.

Two of the major enzymes directly involved in the detoxification of ROS are SOD, scavenging superoxide anion, and GPx, that reduce H_2O_2 and organic hydroperoxides [5]. An increase in the normal levels of SOD was observed in animals treated with vitamin C plus E (100 mg kg^{-1} each), β-carotene (50 mg kg^{-1}), Vimang® (250 mg kg^{-1}) or mangiferin (50 mg kg^{-1}) (Table I). A similar increase was observed also in humans after vitamin supplementation [31]. These effects probably occur thought redox-induced modulation of gene expression to SOD [32, 33]. TPA also increases SOD levels, and such effects could be ascribed to the interaction of TPA with the activator protein-1 (AP-1) DNA binding site (also known as TPA response element). The binding to the AP-1 DNA domains ultimately leads to the expression of a series of genes involved in cell growth and development [34]. The groups pretreated with Vimang® or mangiferin after TPA-induction have the highest levels of SOD, suggesting a synergic effect on SOD activity.

After TPA administration a significant decrease in GPx activities is evident in blood samples (Table I). These results agree with the proposed hypothesis that ROS could oxidize critical protein sulfhydryl groups [35]. With the exception of mangiferin (50 mg kg^{-1}), all the antioxidants tested attenuated the increase in GPx levels, and only in the animals pretreated with the highest dose of Vimang® was the normal level of GPx reached.

The measurement of cytochrome c reduction and H_2O_2 levels (Table II) assessed the production of ROS by peritoneal macrophages. Cytochrome c reduction is a specific test for superoxide anion production [27], whereas H_2O_2 is derived from the dismutation of superoxide anion. These assays clearly demonstrated the production of ROS by peritoneal macrophages after administration of TPA and the comparative protective abilities of Vimang®, mangiferin, vitamin C, a combination of vitamin C plus vitamin E and β-carotene. There was a linear correlation between the values of cytochrome c reduction and the H_2O_2 levels (0.89). Vimang® demonstrated the best protection in both assays as compared to the vitamins, β-carotene, or mangiferin, at the experimented doses. In addition to the abilities of mangiferin to scavenger superoxide anion [25], the amentoflavone present in Vimang® was demonstrated to have a potent inhibitory effect on degranulation of inflammatory cells [29]. This synergic effect may explain the effectiveness of Vimang® in those assays. These data indicated that Vimang® might be useful in preventing the *in vivo* production of ROS as well as other antioxidants.

Oxidative damage to proteins is a very important aspect related to *in vivo* conditions, either on its main function (affecting the function of receptors, enzymes, transport protein, etc. and perhaps generating new antigens that provoke immune response) or on the possibility to contribute to secondary damage on other biomolecules [5]. Sulfhydryl oxidation is one of the earliest observable events during the radical-mediated oxidation of protein [36]. In liver homogenates, only in mice pretreated with vitamin C plus E, mangiferin or Vimang® could the sulfhydryl group reach normal levels after TPA stress induction (Table III). Vimang® has previously demonstrated [37] its strong ability to protect *in vitro* the OH•-mediated oxidation to bovine

serum albumin (BSA). In those experiments, the extract was effective in reducing the oxidation of BSA, since its half-maximal inhibition concentration was 0.0049% w/v in the reduction of carbonyl groups formation and 0.0008% w/v in the inhibition of sulfhydryl groups loss. The protection of OH•-mediated oxidation of BSA takes place essentially by reducing the H_2O_2 concentration, a fundamental component in Fenton-type reactions, by chelating iron or by scavenging the OH• radical formed on the immediate oxidation site on the target protein [38]. Vimang® was able to scavenge OH• (0.011% w/v) and chelating iron (0.117% w/v) according to a collateral report [8]. The present experiment demonstrated the correlation between *in vitro* and *in vivo* assays with respect to the abilities of Vimang® to protect proteins against oxidative damage.

Fragmentation of nuclear DNA is a biochemical hallmark of apoptosis [39]. With the exception of vitamin C in liver homogenates, all the tested antioxidants ameliorated TPA-induced increase in DNA fragmentation in both liver and brain tissues (Table IV). Combination of vitamin C plus vitamin E and Vimang® showed the best protection as compared to the other antioxidants. DNA fragmentation during apoptosis occurs essentially because of an increase in the level of Ca^{2+}, stimulating endonuclease activity with the consequence of an enzymatic fragmentation of genomic DNA [20]. Therefore, the protection mechanism of antioxidants is suggested to work by maintenance of Ca^{2+} homeostasis and cellular integrity.

LP is probably the most extensively investigated process induced by free radicals. The abundant presence of lipids at sites where radicals in general and, more specifically, ROS are formed render them easily accessible as endogenous targets [40]. Biomarkers of LP were assessed in serum, hepatic mitochondria and microsomes and in brain tissues (Table V). Combinations of vitamins, vitamin E, mangiferin and Vimang® as compared to the other antioxidants obtained the best protection against LP. These results are in accordance with other reports on the *in vitro* inhibition of LP by Vimang® in rat brain phospholipid and in rat hepatic microsomes [8, 37]. In the same reports Vimang® inhibits LP with a low half-inhibition concentration. Mangiferin is a potent inhibiting agent of LP as demonstrated performing *in vivo* assays [41, 42]. In Vimang®-treated mice mangiferin represents the main component in LP inhibition.

An additional element that may contribute to the antioxidant mechanism of Vimang® is organic selenium. At Vimang® dose of 250 mg kg^{-1} the corresponding content of organic selenium is 0.125 mg kg^{-1}. Our data demonstrated that Vimang® (250 mg kg^{-1}) was the only substance capable of maintaining GPx at its normal levels after the TPA-oxidative stress induction (Table I). Selenium has been recognized as an essential nutrient on the basis of its ability to serve interchangeably with vitamin E in the prevention of vascular or muscular signs in experimental animals [43]. In addition, selenium is an essential component of seleno-enzymes (GPx and thioredoxin reductase) that play an important role in the antioxidant protection of cells [44].

These *in vivo* experiments have demonstrated that Vimang® is a better scavenger of ROS and inhibitor of oxidation tissue damage than vitamin C, vitamin E, a combination of vitamin C plus vitamin E, mangiferin or β-carotene in the assayed experimental conditions. The results clearly demonstrate that Vimang® significantly attenuates the TPA-induced oxidative stress in serum, hepatic and brain tissues, as well as in peritoneal exudate. Furthermore, the results indicate that Vimang® has a high bioavailability to vital target organs, including liver and brain tissues, serum and peritoneal exudate cells, and therefore may be useful to prevent the production of ROS and oxidative tissue damage *in vivo*.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support from the Pharmacology Laboratory, Centre of Pharmaceutical Chemistry (Havana, Cuba) and the technical assistance of Yaitsé Cruz and Juliette Escoto. This work was funded partially by Project 004003030, Ministry of Science, Technology and the Environment (CITMA), Havana, Cuba and the Italian MURST (Funds 1999 given to LR).

REFERENCES

1. McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radic Biol Med* 1999; **26:** 1034–53.
2. Maxwell SRJ. Antioxidant therapy: does it have a role in the treatment of human disease? *Expert Opin Invest Drugs* 1997; **6:** 211–36.
3. Campo GM, Squadrito F, Campo S, Altavilla D, Avenoso A, Ferlito M, Squadrito G, Caputi AP. Antioxidant activity of U-83836E, a second generation lazaroid, during myocardial ischemia/reperfusion injury. *Free Radic Res* 1997; **27:** 577–90.
4. McCord JM. Human disease, free radicals and the oxidant/antioxidant balance. *Clin Biochem* 1993; **26:** 351–7.
5. Diplock AT, Charleux JL, Crozier-Willi G, Kok FG, Rice-Evans C, Roberfroid M, Stahl W, Viña-Ribes J. Functional food science and defense against reactive oxidative species. *Br J Nutr* 1998; **80(Suppl. 1):** S77–112.
6. Aruoma O. Extracts as antioxidant prophylactic agents. *Inform* 1997; **8:** 1236–41.
7. Centre of Pharmaceutical Chemistry, 1998. Pharmaceutical compositions including a mixture of polyphenols, terpenoids, steroids, fatty acids and microelements with antioxidant, analgesic, anti-inflammatory and anti-spasmodic properties. Patent pending 203/98, OCPI. Havana, Cuba.
8. Sánchez GM, Delgado R, Nuñez-Sellés AJ, Davison GP, Garrido G, León OS. Evaluation of the *in vitro* antioxidant activity of *Mangifera indica* L. extract (Vimang®). *Phythother Res* 2000; **14:** 424–7.
9. Cholbi MR, Paya M, Alcaraz MJ. Inhibitory effect of phenolic compounds on CCL4-induced microsomal lipid peroxidation. *Experientia* 1991; **472:** 195–9.
10. Sato TAK, Tamura A, Tatsum Y, Fuiji T. Mechanism of antioxidant action of *Pueraria* glycoside (PG)-1 (an isoflavonoid) and Mangiferin (a xanthonoid). *Chem Pharm Bull* 1992; **40:** 721–4.
11. Hsu MF, Raung SL, Tsao LT, Lin CN, Wang JP. Examination of the inhibitory effect of norathyriol in formylmethionyl-Leucyl-

Phenylalanine-induced respiratory burst in rat neutrophils. *Free Radic Biol Med* 1997; **23:** 1035–45.
12. Witz G, Czerniecki BJ. Tumor promoters differ in their ability to stimulate superoxide anion radical production by murine peritoneal exudate cells following *in vivo* administration. *Carcinogenesis* 1989; **10:** 807–11.
13. Bagchi M, Stohs JJ. *In vitro* induction of reactive oxygen species by 2,3,7,8-tetrachlorodibenzo-p-dioxin, endrin and lindane in rat peritoneal macrophages and hepatic mitochondria and microsomes. *Free Radic Biol Med* 1993; **14:** 11–8.
14. Sedlak J, Lidsay RH. Estimation of total protein bound and nonprotein sulfhydryl group in tissue with Ellman's reagent. *Anal Biochem* 1968; **25:** 192–205.
15. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation product: Malonaldehyde and 4-hydroxynonenal. *Methods Enzymol* 1990; **186:** 407–21.
16. Ozdemirler G, Mehmetcik G, Oztezcan S, Toker G, Sivas A, Uysal M. Peroxidation potential and antioxidant activity of serum in patients with diabetes mellitus and myocard infarction. *Horm Metab Res* 1995; **27:** 194–6.
17. Spector T. Refinement of the coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 microgram of protein. *Anal Biochem* 1978; **86:** 142–6.
18. Babior BM, Kipner RS, Cerutte JT. The production by leukocytes of superoxide: a potential bactericidal agent. *J Clin Invest* 1993; **52:** 741–4.
19. Gibb RK, Taylor DD, Wan T, O'Conor DM, Doering DL, Gercel-Taylor C. Apoptosis as a measure of chemosensitivity of cisplatin and taxol therapy in ovarian cancer cell lines. *Gynecol Oncol* 1997; **65:** 13–22.
20. Ray SD, Kamendulis LM, Gurule MW, Yorkin RD, Corcoran GB. Ca^{2+} antagonists inhibit DNA fragmentation and toxic cell death induced by acetaminophen. *FASEB J* 1993; **7:** 2280–6.
21. Bagchi D, Garg A, Krohn RL, Bagchi M, Bagchi DJ, Balmoori J, Stohs SJ. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. *Gen Pharmacol* 1998; **30:** 771–6.
22. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. *JAOCS* 1998; **75:** 199–212.
23. Rice-Evans CA, Miller NJ, Paganda G. Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radic Biol Med* 1996; **20:** 933–56.
24. Jovanovic SV, Steenken S, Tosic M, Marjanovic B, Simic MG. Flavonoids as antioxidants. *J Am Chem Soc* 1994; **116:** 4846–51.
25. Rouillard LF, Thiais AJ, Robin JR. Cosmetic or pharmaceutical composition containing, as active ingredient, mangiferine or its derivatives, in pure or in plant extracts. *US Patent* 1998; **5:** 824, 320.
26. Markus B. Electrochemical behaviour and antioxidant activity of some natural polyphenols. *Helv Chim Acta* 1996; **79:** 1147–58.
27. Ritchey EE, Wallin JD, Shah SV. Chemiluminescence and superoxide anion production by leukocytes from chronic hemodialysis patients. *Kidney Int* 1981; **19:** 349–58.
28. Bucar F, Jackak SM, Noree Y, Kartnig T, Perera P, Bohlin L, Schubart-Zsilavecz M. Amentoflavone from Biophyttum sensitivum and its effect on COX-1/COX-2 catalysed prostaglandin biosynthesis. *Planta Med* 1998; **64:** 373–4.
29. Tordera M, Ferrándiz ML, Alcaraz MJ. Influence of anti-inflammatory flavonoids on degranulation and arachidonic acid release in rat neutrophils. *Z Naturforsch* 1994; **49C:** 235–40.
30. Halliwell. How to characterise a biological antioxidant. *Free Radic Res Commun* 1990; **9:** 1–32.
31. McCusker CA, Fitz GSP. The effect of vitamin supplementation on antioxidant enzymes. In: *International Conference of Natural Antioxidant and Lipid Peroxidation in Atherosclerosis and Cancer* (ALPAC) 1995; August 22–24, Helsinki, Finland.
32. Fridovich I. Superoxide anion radical ($O_2\cdot$), superoxide dismutase, and related matters. *J Biol Chem* 1997; **272:** 18519–17.
33. Jourd'hevil D, Morise Z, Conner EM, Grisham MB. Oxidants, transcription factors, and intestinal inflammation. *J Clin Gastroenterol* 1997; **25(Suppl. 1):** S61–72.
34. Karin AP. The role of Jun, Fos and AP-1 complex in cell proliferation and transcription. *Biochim Biophys Acta* 1991; **1070:** 129–57.
35. Kedderis GL. Biochemical basis of hepatocellular injury. *Toxicol Pathol* 1996; **24:** 77–83.
36. Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. *Biochem J* 1997; **324:** 1–18.
37. Sánchez GM, Giuliani A, León-F OS, Davison GP, Núñez-Sellés AJ. Effect of *Mangifera indica* L. Extract (Vimang®) on proteins and hepatic microsomes peroxidation. *Phythother Res* 1999a, in press.
38. Kingu CS, Wei R. Alcohol dehydrogenase catalysed reduction of proteincarbonyl derivatives. *J Biochem Biophys Methods* 1997; **34:** 61–8.
39. Kinloch RA, Treherne JM, Furness LM, Hafimohamadreza Y. The pharmacology of apoptosis. *Trends Pharmacol Sci* 1999; **20:** 35–42.
40. Zwart L, Meerman JHN, Commandeur NM, Vermeulen PE. Biomarker of free radical damage applications in experimental animals and in humans. *Free Radic Biol Med* 1999; **26:** 202–26.
41. Born M, Carrupt PA, Zini R, Brée F, Tillement JP, Hostettmann K, Testa B. Electrochemical behaviour and antioxidant activity of some natural polyphenols. *Helv Chim Acta* 1996; **79:** 1147–58.
42. Ghosal S, Rao G, Saravanan V, Misra N, Rana D. A pausible chemical mechanism of the bioactives of mangiferin. *Indian J Chem* 1996; **35B:** 561–6.
43. Schwarz K, Bieri JG, Briggs GM, Scott ML. Prevention of exudative diathesis in chicks by factor 3 and selenium. *Proc Soc Exp Biol Med* 1957; **95:** 621–5.
44. Combs GF, Gray WP. Chemopreventive agents: selenium. *Pharmacol Ther* 1998; **79:** 179–92.