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RESEARCH ARTICLE

In vivo acute toxicological studies of an antioxidant extract from *Mangifera indica* L. (Vimang)

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Abstract
Mango (*Mangifera indica* L.) stem bark aqueous extract (MSBE) is a natural product with antioxidant, anti-inflammatory, analgesic, and immunomodulatory effects. Its formulations (e.g., tablets, capsules, syrup, vaginal oval, and suppositories) are known by the brand name of Vimang®. In view of the ethnomedical, preclinical, and clinical uses of this extract and the necessity to assess its possible toxicological effect on man, a toxicological analysis of a standard extract is reported in this paper. Acute toxicity was evaluated in mice and rats by oral, dermal, and intraperitoneal (i.p.) administration. The extract, by oral or dermal administration, showed no lethality at the limit doses of 2,000 mg/kg body weight and no adverse effects were found. Deaths occurred with the i.p. administration at 200, but not 20 mg/kg in mice. MSBE was also studied on irritant tests in rabbits, and the results showed that it was nonirritating on skin, ocular, or rectal mucosa. The extract had minimal irritancy following vaginal application.

Keywords: Mangifera indica *L.; Acute toxicity; Irritation; Rat; Rabbit; Mice*

Introduction

Mangifera indica L. (Anacardiaceae) grows in tropical and subtropical regions of the world, and its parts are commonly used in folk medicine for a wide variety of remedies (Coe and Anderson, 1996). The chemical composition of this plant has been studied for its concentration of triterpenes, flavonoids (e.g., polyphenols), and phytosterols (Anjaneyulu et al., 1994; Khan et al., 1994).

In Cuba, the aqueous extract of *Mangifera indica* L. (MSBE) is used on patients suffering from elevated stress (Guevara et al., 2002) as a nutraceutical product. MSBE chemical composition has been reported elsewhere (Núñez et al., 2002), which has enabled the isolation and identification of phenolic acids (e.g., gallic acid, 3,4 dihydroxy benzoic acid, and benzoic acid), phenolic esters (e.g., gallic acid methyl ester, gallic acid propyl ester, and benzoic acid propyl ester), flavan-3-ols (e.g., catechin and epicatechin), and the xanthone, mangiferin. Mangiferin is the major component of MSBE (10–20%), with a typical isomeric composition (mangiferin + isomangiferin + homomangiferin) different from other mangiferins extracted from other regions or natural sources. Biologically active terpenoids, such as beta-elemene, beta-selinene, alpha-guaiene, hinesol, and beta-eudesmol, have been also identified. Other components, such as free sugars, polyalcohols, sterols, and unsaturated fatty acids (e.g., myristic, palmitic, stearic, oleic—linoleic, and eicosatrienoic) have been also reported and quantified. The elemental composition of MSBE by induced coupled plasma (ICP) spectrometry has been reported recently, with Ca, Mg, K, and Fe as the main components, and Cu, Zn, and Se as the microelements. These were related to varietal difference and plant age. Toxic elements (e.g., As, Cd, and Hg) were not detected, and Pb was

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below the allowable amount for human consumption (0.07 mg/100 mL in plasma) (Núñez et al., 2007).

MSBE has antioxidant effect both *in vitro* and *in vivo* (Martinez et al., 2000a, 2000b, 2001). The preclinical pharmacological studies have showed that MSBE has a powerful scavenger activity on hydroxyl radicals and hypochlorous acid, presented a significant inhibitory effect on the peroxidation of rat-brain phospholipid, and inhibited DNA damage by bleomycin or copper-phenanthroline assays. It has been shown that MSBE induced protection against Fe^{2+}-citrate-induced mitochondrial swelling and loss of mitochondrial transmembrane potential. The IC_{50} value for MSBE protection against Fe^{2+}-citrate-induced mitochondrial thiobarbituric reactive substances (TBARSs) formation was around 10 times lower than that for *tert*-butylhydroperoxide. The extract also inhibited the iron citrate induction of mitochondrial antimycin A—insensitive oxygen consumption, stimulated oxygen consumption due to Fe^{2+} autoxidation, and prevented Fe^{3+} ascorbate reduction. The extract polyphenolic compounds, mainly mangiferin, could form a complex with Fe^{2+}, accelerating its oxidation, and the formation of more stable Fe^{3+}-polyphenol complexes, unable to participate in Fenton-type reactions and the lipoperoxidation propagation phase (Andreu et al., 2005). The strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity suggests that besides its iron-complexing capacity, MSBE could also protect mitochondria from Fe^{2+}-citrate lipoperoxidation through direct free radical scavenging ability, mainly lipoperoxyl and alcoxyl radicals, acting as both a chain-breaking and iron-complexing antioxidant (Pardo-Andreu et al., 2006a). MSBE effects on the degradation of 2-deoxyribose induced by Fe^{3+}-ethylenediaminetetraacetic acid (EDTA) plus ascorbate or plus hypoxanthine/xanthine oxidase was studied. The extract was shown to be a potent inhibitor of 2-deoxyribose degradation mediated by Fe^{3+}-EDTA plus ascorbate or superoxide (Pardo-Andreu et al., 2006b, 2006c).

MSBE has also anti-inflammatory, analgesic, and immunomodulatory effects in different experimental models (Garrido et al., 2001; 2004a, 2004b; 2005; 2006; Garcia et al., 2002; Leiro et al., 2004).

MSBE is the pharmaceutical active ingredient used in several formulations, such as coated tablet, gelatin capsule, syrup, cream, ointment, suppository, vaginal oval, and ampoule for injection (which have been protected by a patent; Center of Pharmaceutical Chemistry, 2002) and registered as a phytodrug, food supplement, or cosmetic by the Cuban health regulatory agencies under the brand name of Vimang®.

Although *M. indica* has been used extensively in folk medicine and diverse studies on the biological activities of MSBE have been performed, no detailed toxicological assessment of this extract has been reported. Accordingly, as part of the safety evaluations of MSBE and Vimang´s formulations, acute (i.e., oral, i.p. and dermal toxicity) and irritability (i.e., dermal, ocular, rectal, and vaginal) studies were conducted.

Materials and methods

Plant material

Mango stem bark (*Mangifera indica* L.) was collected from a cultivated field located in the region of Pinar del Rio, Cuba (cultivar M11). Archived specimens of the plant (Code: 41722) were deposited at the Herbarium of the Academy of Sciences, guarded by the Institute of Ecology and Systematic, Ministry of Science, Technology and Environmental (La Habana, Cuba). MSBE was prepared by decoction for 1 h. The extract was concentrated by evaporation and then spray-dried to obtain a fine brown powder (MSBE), which is used as the standardized active pharmaceutical ingredient of Vimang formulations. It melts at 210–215°C with decomposition. The chemical composition of MSBE has been characterized by chromatographic (i.e., planar, liquid, and gas) methods, mass spectrometry, nuclear magnetic resonance (NMR), and UV-V spectrophotometry (Núñez et al., 2002). The elemental inorganic composition has been determined by ICP spectrometry (Núñez et al., 2007).

Animals

New Zealand White rabbits were from Centro para la Producción de Animales de Laboratorio (CENPALAB; La Habana, Cuba), weighing 2.5–3.0 kg at the start of the studies of irritability and approximately 16–24 weeks of age, were acclimatized for at least 5 days prior to the study. The animals were individually housed in suspended metal cages and had free access to food and water throughout the study. The animal rooms were maintained at 20 ± 3°C and relative humidity of 40–70% under a 12-h light-dark cycle.

Sprague-Dawley rats (160–220 g) and OF-1 mice (25–30 g) were from CENPALAB and acclimatized to the environmental conditions for 1 week before the tests. In each case, 3 animals per sex were used. Animals were housed in plastic and stainless-steel grid-floored cages, which were kept at 23 ± 2°C, relative humidity 40–70%, and 12-h light-dark cycles with food and water *ad libitum*.

Experimental protocols

All study protocols were in compliance with Good Laboratory Practice (GLP) standards. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee for Animal Experimentation of the Center of Pharmaceutical Chemistry.

Acute oral toxicity study
The test material was administered once orally via gastric intubation to a mice and rats at a single dose level of 2,000 mg/kg body weight, according to the ATC method OECD Protocol 423 (2000a). MSBE was suspended in carboxymethyl cellulose 0.5% and administered at 10 mL/kg body weight for mice or 5 mL/kg body weight for rats.

Acute dermal toxicity study
MSBE was dissolved in water and administered dermally (0.100 mL) as a single dose of 2,000 mg/kg body weight on the dorsal region of each mouse and rat in a shaved area of 2 cm^2, according to the guideline OECD Protocol 434 (2004).

Acute i.p. toxicity study
In this study, the dose for mice and rats was selected according to the ATC method (OECD, 2000a). The administered starting MSBE dose was 2,000 mg/kg body weight following a second (200 mg/kg body weight) and a third (20 mg/kg body weight) doses dissolved in Tween 20 (0.4% in distilled water).

Observation of clinical signs
These signs were registered as time, duration, and intensity. The animals were observed during the 1-, 2-, 4-, and 6-h postadministration. During the remaining 14 days, each animal was daily observed for the detection of symptoms. Appearance and overt behavior were recorded, so that change in the skin and fur, eyes, and mucus membranes, as well as any disturbances on the respiration, circulation, autonomic or central nervous system, and behavior pattern, were observed. Body-weight data were measured at the beginning and the end of the study. Also, the incidence of gross pathological changes observed during the necropsy performed at Day 14 was included in each study.

Acute dermal irritation study in rabbits
The acute dermal irritation study was performed to assess the potential irritancy of MSBE following a single application to intact rabbit skin, according to the OECD guideline 404 (2000b) and Matulka et al. (2004).

Approximately 24 h prior to the start of testing, 3 rabbits were clipped free of fur from the dorsal-flank area (10 x 10 cm), using veterinary clippers. Only animals with a healthy, intact epidermis were utilized for the study. At the start of the study, 0.5 mL of MSBE 1.2% was placed onto the shorn skin and two 2.5 x 2.5 cm gauze patches were placed over the treated area. Each patch was secured in position with a strip of surgical adhesive tape. The trunk of each rabbit was then wrapped and the animals returned to the cages for the duration of the exposure period. Four h after the application, the patches were removed from each animal and residual test material removed with cotton wool soaked in diethyl ether. One h following the removal of the patches and approximately 24, 48, and 72 h after patch removal, the test sites were examined for evidence of primary irritation and scored, according to Draize et al. (1944). The scores for erythema and edema at the 24-, 48- and 72-h readings were totaled for the 3 test rabbits and were then divided by 12 to give the primary irritation index of the test material.

Acute ocular irritation study in rabbits
The acute ocular irritation study was performed to assess the potential irritancy of MSBE 1.2% following a single application to the rabbit eye, according to the OECD guidelines (2000b) and Matulka et al. (2004). On the day prior to the study, both eyes of 3 selected test rabbits were examined under ultraviolet light, after treatment with sodium fluorescein B.P. (Fluorets; Smith & Nephew Pharmaceuticals, London, UK). The cornea, conjunctiva, and iris were examined for lesions. Immediately before treatment, the rabbit eyes were again examined with the aid of a light source and any animals showing evidence of ocular lesions were rejected and replaced. To minimize pain on instillation of the test material, 1 drop of local anesthetic (proxymetacaine hydrochloride 0.5%; Squibb & Sons, Houndslow UK) was added to both eyes, 1–2 min before test-material treatment. A volume of 0.1 mL of the test material was instilled into the conjunctiva of the right eye of each animal, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about 30 s immediately after instillation, to prevent loss of the test material, and then released. The left eye remained untreated and was used for control purposes.

Assessment of ocular damage/irritation was made at approximately 1, 24, 48, and 72 h following treatment, according to the numerical evaluation given in Appendix I of Draize et al. (1944). All observations were registered to calculate the ocular Irritation Index (OII). The OII was calculated by adding all injuries found in the 3 analyzed structures (e.g., cornea, iris,

and conjunctiva) in each time and this value was divided by 12. Any other ocular effects were also noted. Examination of the eye was facilitated by use of a standard ophthalmoscope.

Rectal irritation study in rabbits

Studies were conducted to determine the effect of MSBE on its irritation potential for the rectal mucosa of the albino rabbit.

MSBE (1.2%) and placebo (carboxymethylcellulose 0.5%) were administered intrarectally by lavage (2 mL) once a day for 5 consecutive days. A group without treatment was used as control. The rabbits were observed each day before and after treatment for rectal changes.

On Day 6, animals were humanely killed, and parts of ani and rectum (10 cm) of each animal were observed macroscopically and then fixed in 10% neutral-buffered formalin. Fixed anal and rectal tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Stained sections were examined by light microscopy by a board-certified veterinary pathologist. It was evaluated by the inflammation grade and altered superficies. According to the reactions of epithelium, leukocyte inflammation, vascular congestion, and edema, a histological study was performed to know the anatomo-pathological origin of injury. A compared irritation index (i.e., irritation index of treated group–irritation index of control group) was calculated.

Vaginal irritation study in rabbits

Studies were conducted to determine the effect of the MSBE vaginal douche on its irritation potential for the vaginal mucosa of the albino rabbit, using the technique of Kaminsky and Willigan (1982). The MSBE preparations (0.5 and 1.0%) and placebo (saline solution 0.9%) were administered intravaginally by lavage (2 mL) once a day for 5 consecutive days. A group without treatment was used as the control. The animals were observed each day before and after treatment, searching any vaginal abnormality. On Day 6, animals were humanely sacrificed, and parts of the cervicovagina, midvagina, and urovagina of each animal were fixed in 10% neutral-buffered formalin. Fixed vaginal tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Stained sections were examined by light microscopy by a board-certified veterinary pathologist.

Each of the 3 regions of vagina was scored blindly for epithelial ulceration, leukocyte infiltration, edema, and vascular congestion. The irritation scores were assigned based on the semiquantitative scoring system of Eckstein et al. (1969),which was as follows: individual score, 0 = none; 1 = minimal; 2 = mild; 3= moderate; and 4 = intense irritation. This scoring system correlates to human irritation potential (compared) as follows: scores of 0 to 8 are acceptable; scores of 9 to 10 indicate borderline irritation potential; and scores of 11 and above are indicative of significant irritation potential.

Results and discussion

This paper reports the results of preliminary toxicity evaluations of MSBE, which is the active pharmaceutical ingredient of Vimang´s formulations (e.g., tablets, capsules, syrup, vaginal oval, suppositories, etc.) used in Cuba as an antioxidant (Martinez et al., 2000a, 2000b; 2001), anti-inflammatory, analgesic, and immunomodulator extract (Garrido et al., 2001, 2004a, 2004b, 2005, 2006; Garcia et al., 2002; Leiro et al., 2004).

Acute toxicity studies represent the first step of the preclinical development of a new product with therapeutic potential.

The consumer products industry is concerned with developing not only safe products, but products that are milder and more efficacious.

No deaths were registered after the administered MSBE dose (2,000 mg/kg) in both sexes in rats and mice after a single oral dose. Only transitory piloerection was observed in the mice treated with the product during the first hour after the exposure; no other clinical symptoms were observed. No gross histopathological alterations were found at necropsy of the animals at the end of the study.

In a dermal acute toxicity test, there were no deaths, test material–related clinical findings, other local reaction to treatment, or remarkable body-weight changes after acute dermal administration of MSBE to mice or rats at 2,000 mg/kg body weight.

Table 1 shows deaths that occurred during an i.p. acute toxicity assay, all occurring within 72 h of treatment. Many signs of response were observed in the animals prior to their death, including decreased motor activity, prostration, piloerection, ataxia, dyspnea, pigmented orbital secretion, and diarrhea.

The irritant capability of MSBE 1.2% on rabbit dermis was examined. There were no erythema or edema recorded 24, 48, and 72 h after treatment,the irritation index was 0.0, and the material was considered nonirritating.

An acute eye irritation study with MSBE was performed. Corneal damage in 1 treated rabbit at 24 h, that disappeared at 48 h after MSBE application, was seen. There were no conjuctival or iridial alterations during the study. The OII to MSBE 1.2% was 0.42.

Table 1. Mortality of mice and rats after i.p. administration with a single dose of MSBE.

	Mice		Rats		
Dose (mg/kg)	Male	Female	Male	Female	Clinical signs[a]
0	0	0	0	0	Decreased motor activity,
20	0	0	0	0	prostration, piloerection, ataxia,
200	2	1	1	2	Dyspnea, pigmented orbital,
2000	3	3	3	3	secretion and diarrhea

[a]Only in those animals that died. All the deaths happened in the first 72 h. F, female; M, male. MSBE, *Mangifera indica* stem bark extract.

Table 2. Rectal and vaginal irritation index (individual and compared) for New Zealand rabbits given MSBE intra-rectally or vaginally for 6 days.

	Irritation index	
Treatment	Individual	Compared
Rectal Irritation		
Control	0.83	0.0
Placebo	1.41	0.58
MSBE 1.2%	1.36	0.53
Vaginal Irritation		
Control	3.3	0.0
Placebo	3.3	0.0
MSBE 0.5%	6.6	3.3
MSBE 1.0%	7.6	4.3

MSBE, *Mangifera indica* stem bark extract.

The study to determine the rectal irritancy of MSBE 1.2% was also performed in rabbits. No damage was seen following macroscopic evaluation. The microscopy study showed minimal types of reaction (shown on Table 2), with individual or irritation compared indices of 1.36 and 0.53, respectively.

During the experimental 6 days, observations of vaginal opening for searching erythema, edema, or any other damage were performed. There were no clinical signs of alterations in the animals under the conditions of this study. Table 2 lists the individual irritation index vs. the compared index. As can be seen, the group of MSBE 0.5% showed an irritability of minimal grade, characterized by generalized erosion and moderated grade of inflammatory cells infiltration in two animals. In the group of MSBE 1.0%, the irritability was of minimal grade, although there was severe damage characterized by generalized erosion, moderated vascular congestion, leukocyte infiltration, and moderated edema in a rabbit. Since a correlation exists between rabbits and humans with respect to the irritation potential of vaginal products, these findings indicated that the irritation potential of MSBE is well below the acceptable range (total score =<8) for the clinical trial. Thus, MSBE is not likely to cause mucosal toxicity following repetitive intravaginal applications in humans. However, the administration of Vimang vaginal oval should be applied with caution because repeat administrations may not cause irritation, but the histopathological changes could lead to an increase in fibrosis or scar tissue.

Conclusions

The extract of MSBE, by acute oral or dermal administration, showed no lethality at the limit doses of 2,000 mg/kg body weight and no adverse effects were seen. Deaths occurred with the i.p. administration at 200, but not at 20 mg/kg in mice and rats. The extract was also studied on irritant testes in rabbits and the results showed that it was nonirritating on skin, ocular, or rectal mucosa. The extract had minimal irritancy following vaginal application.

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References

Andreu GL, Delgado R, Velho J, Curti C, Vercesi AE (2005). Iron complexing activity of mangiferin, a naturally occurring glucosylxanthone, inhibits mitochondrial lipid peroxidation induced by Fe2+-citrate. *Eur. J. Pharmacol.* 513, 47–55.

Anjaneyulu V, Babu IS., Connolly JD (1994). 29-hydroxymangiferonic acid from *Mangifera indica*. *Phytochemistry* 35, 1301–1303.

Center of Pharmaceutical Chemistry. (2002). Pharmaceutical composition including a mixture of polyphenols, terpenoids, steroids, fatty acids, and microelements with antioxidant, analgesic, anti-inflammatory, and antispasmodic properties. Cuban Patent 203/98, OCPI, Havana, Cuba.

Coe FG, Anderson GL (1996). Screning of medicinal plants used by the Garifuna of eastern Nicaragua for bioactive compounds. *J. Ethnopharmacol* 53, 29–50.

Draize JH Woodward G, Calvery HO (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exper.l Ther.* 82, 377–390.

Eckstein P, Jackson MCN, Millman N, Sobrero AJ (1969). Comparison of vaginal tolerance tests of spermicidal

preparations in rabbits and monkeys. *J. Reprod.Fertil.* 20, 85–93.

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

Garrido G, Gonzalez D, Delpote R, Backhouse N, Quintero G, Nuñez-Selles A (2001). Anagelsic and anti-inflammatory effects of *Mangifera indica* L. extract (VIMANG) *Phytother. Res.* 15, 18–21.

Garrido G, Delgado R, Lemus Y, Rodríguez J, Garcia D, Núñez A (2004a). 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.

Garrido G, Gonzalez D, Lemus Y, Garcia D, Lodeiro L, Quintero G et al. (2004b). *In vivo* and *in vitro* anti-inflammatory activity of *Mangifera indica* L. extract (Vimang®). *Pharmacol. Res.* 50, 143–149.

Garrido G, Blanco-Molina M, Sancho R, Macho A, Delgado R, Muñoz E (2005). An aqueous stem bark extract of *Mangifera indica* (Vimang®) inhibits T cell proliferation and TNF-induced activation of nuclear transcription factor NF-kappaB. *Phytother. Res.* 19, 211–215.

Garrido G, Gonzalez D, Lemus Y, Delporte C, Delgado R (2006). Protective effects of a standard extract of *Mangifera indica* L. (VIMANG®) against mouse ear edemas and its inhibition of eicosanoid production in J774 murine macrophages. *Phytomedicine* 13, 412–418.

Guevara M, Riaño A, Alvarez A, Garrido G, Paez E, Delgado R (2002). *Mangifera indica* L. Uso etnomédico en Cuba. *Rev. Cubana Farmacia* 36, 166–168.

Kaminsky M, Willigan DA. (1982). pH and the potential irritancy of douche formulations to the vaginal mucosa of the albino rabbit and rat. *Food. Cosmet. Toxicol.* 20, 193–196.

Khan MA, Nizami SS, Khan MNI, Azeem SW, Ahmed Z (1994). New triterpenes from *Mangifera indica*. *J Nat. Prod.* 57, 988–991.

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

Martinez G, Delgado R, Perez G, Garrido G, Nuñez A, Leon O (2000a). Evaluation of the *in vitro* antioxidant activity of *Mangifera indica* L. extract (Vimang®). *Phytother. Res.* 14, 424–427.

Martínez SG., Jalil EC, Giuliani A, León FOS, Ram S, Delgado HR et al. (2000b). *Mangifera indica* L. extract (Vimang®) reduces ischaemia-induced neuronal loss and oxidative damage in gerbil brain. *Free Radical Res.* 32, 1–3.

Martínez SG., Giuliani A, León OS, Pérez DG, Núñez AJ. (2001). Effect of *Mangifera indica* L. extract (Vimang®) on protein and hepatic microsoma peroxidation. *Phytother. Res.* 15, 581–585.

Matulka RA, Hood AM, Griffiths JC (2004). Safety evaluation of a natural tomato oleoresin extract derived from food-processing tomatoes. *Regul. Toxicol. Pharm.* 39, 90–402.

Núñez A, Velez H, Aguero-Agüero J, Gonzalez I, Naddeo F, De Simone F et al. (2002). Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyphenols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. *J. Agr. Food Chem.* 50, 762–766.

Nuñez AJ, Durruthy MD, Rodríguez E, Nieto L, Nicolais V, Rastrelli L (2007). Comparison of major and trace element concentration in sixteen varieties of Cuban mango stem bark (*Mangifera indica* L.). *J. Agr. Food Chem.* 55, 2176–2181.

OECD. (2000a). Guideline for the Testing of Chemicals, No. 423. Acute Oral Toxicity–Acute Toxic Class Method. Paris, France: Organization for Economic Cooperation and Development.

OECD. (2000b). OECD Guideline for Testing of Chemicals, No. 404. Acute Dermal Toxicity–Fixed Dose Procedure. Paris, France: Organization for Economic Cooperation and Development.

OECD. (2004). OECD Guideline for Testing of Chemicals, No. 434. Acute Dermal Toxicity–Fixed Dose Procedure. Paris, France: Organization for Economic Cooperation and Development.

Pardo-Andreu G, Delgado R, Núñez-Sellés AJ, Vercesi A E (2006a). *Mangifera indica* L. extract (Vimang®) inhibits 2-deoxyribose damage induced by Fe (III) plus ascorbate. *Phytother. Res.* 20, 120–124.

Pardo-Andreu GL, Sánchez-Baldoquín C, Avila-González R, Suzuki Yamamoto ET, Revilla A, Akira Uyemurad S et al. (2006b). Interaction of Vimang® (*Mangifera indica* L. extract) with Fe(III) improves its antioxidant and cytoprotecting activity. *Pharmacol. Res.* 53, 253–260.

Pardo-Andreu G, Sánchez-Baldoquín C, Ávila-González R, Delgado R, Naal Z, Curti C (2006c). Fe(III) improves antioxidant and cytoprotecting activities of mangiferin. *Eur. J. Pharmacol.* 547, 31–36.