

ORIGINAL ARTICLE

## *Mangifera indica* L. (Vimang) Protection against Serum Oxidative Stress in Elderly Humans

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**Background.** We searched for the protective effect of a natural extract from stem bark of *Mangifera indica* L. extract (Vimang) on age-related oxidative stress.

**Methods.** Healthy subjects were classified in two groups, elderly (>65 years) and young group (<26 years). The elderly group received a daily dose of 900 mg of extract (three coated Vimang tablets, 300 mg each, before meals) for 60 days. Serum concentration of lipid peroxides, serum peroxidation potential, extracellular superoxide dismutase activity (EC-SOD), glutathione status (GSH, GSSG, GSSG/GSH ratio) and total antioxidant status (TAS) were determined before (both experimental groups) and 15, 30, and 60 days after treatment (only elderly group). We confirmed the existence of an age-associated oxidative stress in human serum as documented by an age-related increase in serum lipoperoxides and GSSG and a decrease in serum antioxidant capacity and EC-SOD activity.

**Results.** Vimang tablet supplementation increased EC-SOD activity ( $p < 0.01$ ) and serum TAS ( $p < 0.01$ ). It also decreased serum thiobarbituric reactive substances ( $p < 0.01$ ) and GSSG levels ( $p < 0.05$ ). We suggested that the antioxidant components of the extract could have been utilized by the cells (especially blood and endothelial cells), sparing the intra- and extracellular antioxidant system and increasing serum peroxyl scavenging capacity, thus preventing age-associated increase in GSH oxidation and lipoperoxidation.

**Conclusions.** Vimang tablets prevent age-associated oxidative stress in elderly humans, which could retard the onset of age-associated disease, improving the quality of life for elderly persons. © 2006 IMSS. Published by Elsevier Inc.

**Key Words:** Aging, *Mangifera indica* L., Antioxidants, Oxidative stress, Vimang.

### Introduction

Many theories have been proposed to account for the process of aging. The argument that free radicals cause nonspecific damage to macromolecules such as DNA, lipids, and proteins has been called the free radical theory

of aging (1). This theory has become quite persuasive in recent years due to the development of free radical biology and the growing number of evidences, which have shown that most physiological and biochemical aspects of the aging process correlate with cellular accumulation of oxidized products (2,3).

Vimang is the brand name of an aqueous extract of the mango (*Mangifera indica* L.) stem bark extract (MSBE) of selected varieties, which contains a defined mixture of components including polyphenols, triterpenes, phytosterols, fatty acids and microelements (4,5). Previous experiments

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on that extract have shown that it has antioxidant (6–9), analgesic, and anti-inflammatory (10,11) properties.

Vimang has a powerful *in vitro* scavenger activity of hydroxyl radicals and hypochlorous acid and acts as an iron chelator. MSBE has shown to have a significant inhibitory effect on the peroxidation of rat-brain phospholipids and also inhibited DNA damage by bleomycin or copper-phenanthroline systems (6). It protected mice biomolecules against 12-O-tetradecanoylphorbol-13-acetate-induced oxidation and peritoneal macrophage activation (7), reduced ischemia-induced neuronal loss and oxidative damage in the gerbil brain (8), and prevented liver injury associated with ischemia/reperfusion in rats (9), demonstrating its high antioxidant potential.

Human blood serum contains a variety of antioxidant active biomolecules that can potentially protect circulating lipids, proteins, and cells as well as the vasculature-lining endothelial cells from oxidative damage. For these reasons, together with its ready availability from human subjects, blood serum is a suitable model of extracellular fluids and an ideal biological material to monitor *in vivo* free radical reactions.

The aim of the present study was to investigate the effect of MSBE (as Vimang tablets) on the age-associated oxidative stress by measuring several biochemical markers in serum of elderly people in order to assess its effectiveness as an antioxidant supplement.

## Materials and Methods

### Subjects

Fifty healthy volunteers were classified in two groups, elderly ( $n = 30$ ; >65 years old) with a mean age of 78 years (range 65–96 years) and young ( $n = 20$ ; <26 years), with a mean age of 22 years (range 19–26 years). All subjects were diagnosed as clinically healthy at the time of the study as determined by a medical history questionnaire, physical examination, and normal results of clinical laboratory tests (hemoglobin, hematocrit, leukocyte, glucose, urea, creatinine, urate, total protein, albumin, cholesterol, triglycerides, and high-density lipoprotein levels). They fulfilled the following eligibility criteria: 1) no history of cardiovascular, hepatic, gastrointestinal, or renal diseases; 2) no alcoholism; 3) no smoking; 4) no antibiotic or supplemental vitamin and/or mineral use for at least 4 weeks before the beginning of the study. Informed consent was obtained from all participants and/or their first-degree relatives.

The elderly group (self-sufficient and living on their own) received a daily dose of 900 mg of MSBE (three coated Vimang tablets, 300 mg each, before meals) during a period of 60 days. The Ethics Committee of the Faculty of Pharmacy, University of Camagüey, approved the research

protocol for the study, which was conducted in accordance with the guidelines of the Helsinki Declaration.

### Blood Sampling and Preparation

Blood samples (7 mL) were collected by venipuncture early in the morning after a 12-h fasting period at the following times: pre-treatment (both experimental groups), 15, 30, and 60 days after starting the Vimang supplementation (only the elderly group who received the supplementation). They were placed in vacutainer/siliconized test tubes. Serum was isolated immediately and kept under freezing conditions ( $-20^{\circ}\text{C}$ ) until the beginning of analyses.

### Serum Analyses

Total antioxidant capacity was carried out using ABTS<sup>+</sup> (2,2'-azidodiethylbenzothiazolin sulfanate) radical formation kinetics (Randox Laboratories Ltd., Scotland). The presence of antioxidants in serum suppresses the bluish-green staining of the ABTS<sup>+</sup> cation, which is proportional to the antioxidant concentration. Kinetics was measured at 600 nm (12). It was also determined what is generally considered lipid peroxides (TBA reactants) using thiobarbituric acid (TBA) reactions spectrophotometric assay (13). A standard curve was constructed using malondialdehyde (MDA) bisdimethylacetal. To ensure that no lipid oxidation occurs during the assay, BHT [0.01% (v/v) of a 2% stock solution in ethanol] and EDTA (1 mM final concentration) were added to the sample prior to trichloride acetic acid precipitation. For the determination of peroxidation potential (PP), serum was incubated with copper sulfate (2 mM, final concentration) at  $37^{\circ}\text{C}$  for 24 h. PP was estimated by taking the difference between MDA values at 24 and 0 h (14). Serum total glutathione and oxidized glutathione (GSSG) concentrations were measured by the kinetics assay using the glutathione reductase reaction (15). Autooxidation of GSH to GSSG was prevented by addition of N-ethylmaleimide (NEM) to the samples. The GSH content was calculated by subtracting oxidized glutathione from total glutathione content. SOD activities were assayed by a modified pyrogallol autooxidation method (16).

### Statistical Analysis

Data were analyzed using the statistical programs SPSS 9.0. Non-parametric Friedman test for several related samples was used and when asymptotic significance was  $<0.05$ , the changes within the groups were tested using Wilcoxon's signed ranks test (also known as Wilcoxon's pair test).

Mann-Whitney U test was used to estimate statistical differences between two independent groups (young and elderly groups). A  $p < 0.05$  was considered significant. Values were expressed by mean  $\pm$  standard error of mean (SEM).

## Results

### *Effects of Age and Vimang Supplementation on Serum Antioxidant Status*

The total antioxidant test measures the degree of suppression of a stable radical cation by human serum. The results showed an age-dependent decrease of serum TAS ( $p < 0.01$ ). Table 1 shows that serum antioxidant status from the elderly group was 77% of that found in the young group. Vimang supplementation prevented the age-related decrease in serum TAS. It increased serum antioxidant capacity by 32.4, 36.3, and 55.9% for 15, 30, and 60 days after supplementation, respectively, as compared to the elderly TAS mean value before supplementation.

### *Effects of Age and Vimang Supplementation on Serum Lipoperoxidation*

Table 2 shows that the mean serum TBA reactants in elderly, expressed as MDA ( $\mu\text{mol/L}$ ), was higher ( $p < 0.01$ ) as compared to the young control group. Vimang supplementation decreased ( $p < 0.01$ ) elderly serum MDA levels to  $2.73 \pm 0.55$  after 60 days (Table 2), which represented 58.4% of serum TBA reactants levels found in elderly before Vimang supplementation.

Level of serum PP was not different significantly ( $p > 0.05$ ) between young and elderly groups and was not modified by Vimang supplementation (Table 2).

### *Effects of Age and Vimang Supplementation on Serum Glutathione Redox Status*

The age-related changes in total GSH, GSH, and GSSG levels are reported in Table 1. It was observed that total GSH and GSH levels did not change with age, although a significant increase in GSSG levels was observed for the elderly group. Table 1 also reports an age-dependent increase in serum redox index:  $(2[\text{GSSG}]/([\text{GSH}] + 2[\text{GSSG}])) \times 100$  ( $p < 0.05$ ). No significant changes were

observed in serum GSH levels after Vimang supplementation although it decreased the GSSG serum levels in the elderly group. Vimang supplementation also decreased the serum redox index of this group.

### *Effects of Age and Vimang Supplementation on Serum SOD Activity*

SOD activity was measured quantifying the inhibition of pyrogallol autooxidation at 420 nm. Table 1 shows an age-dependent decrease in SOD activity ( $p < 0.05$ ). Vimang supplementation resulted in a significant increase of SOD activities after 15 days, but activity was reduced after 30 and 60 days of treatment. Nevertheless, SOD activities after 30 or 60 days of Vimang supplementation were still higher than those observed before Vimang supplementation (Table 1).

## Discussion

Recent reports suggest that an improvement of antioxidant status in animals, including humans, could retard cellular and tissue damage as well as the manifestation of aging symptoms. It is often assumed that antioxidant nutrients, especially flavonoids and other phenolic compounds, may contribute to the protection afforded by fruits, vegetables and red wines against diseases of aging (17,18).

Chemical characterization of MSBE has enabled the isolation and identification of phenolic acids (gallic acid, 3,4 dihydroxy benzoic acid, benzoic acid), phenolic ester (gallic acid methyl ester, gallic acid propyl ester, benzoic acid propyl ester), flavan-3-ols (catechin and epicatechin), and mangiferin, the latter being the main component of MSBE (20%) (5). Elemental composition demonstrated the presence of selenium and calcium at quantities that afford for a Recommended Daily Allowance. Zinc and copper were also present in MSBE (19). That MSBE composition and the result of pre-clinical evaluation is the basis for

**Table 1.** Effect of age and Vimang supplementation on serum antioxidant status

|                                             | Young              | Elderly (non-supplemented)       | Elderly (supplemented) (days)    |                                  |                                  |
|---------------------------------------------|--------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
|                                             |                    |                                  | 15                               | 30                               | 60                               |
| Total glutathione                           | 451.06 $\pm$ 19.47 | 446.64 $\pm$ 17.24               | 474.48 $\pm$ 15.32               | 452.52 $\pm$ 19.12               | 462.30 $\pm$ 23.04               |
| GSH (ng/mL)                                 | 440.77 $\pm$ 19.47 | 430.66 $\pm$ 10.4                | 461.92 $\pm$ 11.85               | 440.10 $\pm$ 13.65               | 449.18 $\pm$ 15.78               |
| GSSG (ng/mL)                                | 10.29 $\pm$ 2.17   | 15.98 $\pm$ 2.05 <sup>a,*</sup>  | 12.56 $\pm$ 1.82 <sup>b,*</sup>  | 12.42 $\pm$ 2.11 <sup>b,*</sup>  | 13.12 $\pm$ 1.09 <sup>b,*</sup>  |
| [2GSSG/([GSH]+2GSSG)] $\times$ 100          | 4.46 $\pm$ 1.08    | 6.91 $\pm$ 1.01 <sup>a,*</sup>   | 5.16 $\pm$ 0.92 <sup>b,*</sup>   | 5.34 $\pm$ 0.78 <sup>b,*</sup>   | 5.52 $\pm$ 1.02 <sup>b,*</sup>   |
| SOD activity (U/mL/min)                     | 5.64 $\pm$ 1.08    | 3.84 $\pm$ 0.413 <sup>a,*</sup>  | 10.1 $\pm$ 0.498 <sup>b,**</sup> | 7.08 $\pm$ 0.325 <sup>b,**</sup> | 7.01 $\pm$ 0.355 <sup>b,**</sup> |
| Total antioxidant status (mmol of Trolox/L) | 1.31 $\pm$ 0.058   | 1.02 $\pm$ 0.057 <sup>a,**</sup> | 1.35 $\pm$ 0.039 <sup>b,**</sup> | 1.39 $\pm$ 0.057 <sup>b,**</sup> | 1.59 $\pm$ 0.052 <sup>b,**</sup> |

Values are means  $\pm$  SEM.

<sup>a</sup>Represents significant differences between young and elderly non-supplemented groups.

<sup>b</sup>Represents significant differences between elderly non-supplemented and elderly supplemented groups.

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

**Table 2.** Prevention of age-associated serum oxidative stress by Vimang supplementation

|                                                    | Young            | Elderly (non-supplemented) | Elderly (supplemented) (days) |                         |                         |
|----------------------------------------------------|------------------|----------------------------|-------------------------------|-------------------------|-------------------------|
|                                                    |                  |                            | 15                            | 30                      | 60                      |
| TBA reactants ( $\mu\text{mol}$ of MDA/L)          | $3.55 \pm 0.094$ | $4.67 \pm 0.345^{a,**}$    | $3.48 \pm 0.089^{b,**}$       | $3.21 \pm 0.157^{b,**}$ | $2.73 \pm 0.123^{b,**}$ |
| Peroxidation potential ( $\mu\text{mol}$ of MDA/L) | $14.91 \pm 1.01$ | $14.70 \pm 0.593$          | $15.07 \pm 0.449$             | $14.67 \pm 0.742$       | $14.70 \pm 0.553$       |

Values are means  $\pm$  SEM.

<sup>a</sup>Represents significant differences between young and elderly non-supplemented groups.

<sup>b</sup>Represents significant differences between elderly non-supplemented and elderly supplemented groups.

<sup>\*\*</sup> $p < 0.01$ .

using Vimang tablets as a promising supplementation against oxidative stress for elderly people.

In the present study we confirmed the existence of chronic oxidative stress in serum from elderly people. The SOD activities of the healthy volunteers exhibited a decreasing trend with the increase of age (Table 1), which agrees with recent reports (20,21). This finding could suggest that the decay in SOD activity is, at least in part, involved in the age-related oxidative stress.

It has been reported that an appropriate antioxidant/prooxidant balance plays a pivotal role in maintaining health during aging (22). Previous examinations of the total antioxidant status in various tissues and human serum have found it to decrease markedly with age (23–25). This parameter may be a marker of the antioxidant status of the body and may reflect the level of supplementation with antioxidant vitamins and oxidative stress imposed on the organism (26). Our results have shown that serum TAS values were reduced significantly for the elderly group (Table 1).

There is considerable support for the concept that oxygen free radicals and related lipid peroxidation play a key role in the pathogenesis of normal senescence and of age-related chronic degenerative diseases. Malondialdehyde, a product of lipid peroxidation, has been proposed as a possible marker of aging (27,28). The initial TBA reactant concentration in the elderly group was significantly higher than in the young group. Because MDA production is an index of physiological or pathological lipid peroxidation, these results seem to emphasize the fact that aging is accompanied by an increased rate of free radical generation and lipid peroxidation.

Concerning GSH status, Table 1 shows that serum GSH total and GSH levels remained unchanged with age, indicating that membrane transport of GSH into the cells, which plays a critical role in determining serum GSH levels, did not change during aging (29).

Serum GSSG levels and serum redox index increased with age indicating an age-related glutathione oxidation, which agrees with previous reports (30,31).

Glutathione oxidation in aging can be caused by an increased production of oxidative species, a decreased antioxidant capacity, or both. In general terms, aging is

associated with a decrease in the activities of enzymes, which catalyze reactions tending to reduce GSSG, such as glucose-6-phosphate dehydrogenase or glutathione reductase, rather than increasing the activity of those enzymes which favor oxidation of glutathione, such as glutathione peroxidase or transferase (32).

The above-mentioned results are consistent with the interpretation of the free radical theory of aging, which states that there is a generalized oxidation associated with aging, driven by oxygen and nitrogen free radicals endogenously generated during the lifespan, which may contribute to the development of age-associated pathologies.

Certain impairments associated with aging can be prevented by antioxidant administration (17,33,34). Protective abilities of Vimang supplementation on age-related oxidative stress could be assessed in the present study.

SOD activity was first increased after 15 days of Vimang supplementation, probably through redox-induced modulation of gene expression (35,36), and then it was reduced after 30 and 60 days of supplementation, probably because of a downregulation of SOD (37). Previous studies have demonstrated that endogenous antioxidants are subjected to homeostatic control. Exposure to an oxidative stress *in vivo* can lead to compensatory inductions of endogenous antioxidant (38). Addition of high amounts of exogenous antioxidants in the diet or long-term supplementation could also generate a reactive depression of these endogenous antioxidants. Although SOD activity after 60 days of Vimang supplementation still remained higher than the value observed before supplementation, the reduced activity observed after 30 and 60 days alerts us about the adequate dose to be used, high enough to provide antioxidant beneficial effects, but not so high as to produce a reactive depression of endogenous antioxidants.

Serum TAS has been associated with aging and typical age-related diseases such as atherosclerosis, cancer, diabetes mellitus, Alzheimer's disease, and arterial hypertension (39–41). It has also been demonstrated that serum antioxidant capacity is increased by consumption of strawberries, spinach, and red wine in elderly persons (18). These fruits and vegetables are very rich in antioxidant phenolic compounds, which together with vitamin C and urate account for the increase of antioxidant

capacity. Vimang supplementation exhibited beneficial effects on serum elderly TAS because it prevented the age-related decrease of this parameter. This would suggest a more favorable antioxidant/prooxidant balance in aged serum after Vimang treatment, which is therefore likely to be healthier.

Concerning Vimang supplementation effects on serum lipoperoxidation in the elderly, it was observed that after 2 months of Vimang supplementation MDA levels in elderly people decreased to  $2.73 \pm 0.55 \mu\text{mol/L}$  (Table 2), even below the value of the younger controls. These results suggested that Vimang supplementation for elderly people was especially beneficial because it could retard or prevent the onset of many age-related diseases connected with accelerated radical generation and lipid peroxidation.

Table 2 shows that serum PP was not affected by age or Vimang supplementation. It seems that MSBE protection against lipoperoxidation was more effective for a cell-containing system, where it can stimulate enzymatic antioxidant activities such as SOD or glutathione peroxidase (42).

A decrease in GSSG accompanied by no change in total GSH and GSH levels was observed in elderly serum after Vimang supplementation (Table 1). The serum redox index, calculated on the basis of thiol units, also showed a decrease during the 2-month period of Vimang supplementation. Recent reports have shown that pre-treatment of mice with MSBE (250 mg/kg) or mangiferin (50 mg/kg) reduced 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced sulfhydryl loss in supernatant of hepatic homogenate, which was correlated with their strong ability to protect *in vitro* the hydroxyl ( $\text{OH}^\cdot$ )-mediated oxidation to bovine serum albumin (BSA). In those experiments, MSBE was effective by reducing the oxidation of BSA, since its half-maximal inhibition concentration was 0.0008% w/v for the inhibition of sulfhydryl groups loss (7). The protection of  $\text{OH}^\cdot$ -mediated oxidation of BSA takes place essentially by reducing the  $\text{H}_2\text{O}_2$  concentration, a fundamental component in Fenton-type reactions, by chelating iron or by scavenging the  $\text{OH}^\cdot$  radical formed on the immediate oxidation site on the target protein (7). MSBE was able to scavenge  $\cdot\text{OH}$  (0.011% w/v) and chelating iron (0.117% w/v) (6).

The GSSG reduction observed after Vimang supplementation could also be produced by the stimulation in the activities of enzymes by MSBE, which catalyze reactions tending to reduce GSSG, such as glucose-6-phosphate dehydrogenase or glutathione reductase, especially in erythrocytes.

Mangiferin, which accounts for about 20% of MSBE, has been tested *in vitro* for its antioxidant properties (11,43) and recently it was also tested *in vivo* (7). Aglycone of mangiferin (norathyriol) has also been tested *in vitro* as inhibitor of the formylmethionyl-leucyl-phenylalanine-induced respiratory burst in rat neutrophils (44). The second and third main polyphenols in MSBE, catechin

(1.3%) and epicatechin (0.8%), have also been identified as powerful antioxidants, reversing the oxidative damage induced by ozone inhalation in a murine model of oxidative stress (45) and prolonging mean life span in chronically treated senescence accelerated mice (SAM-P8 strain) (46).

Selenium is an essential constituent of a number of enzymes, some of which have antioxidant functions. The benefits of selenium supplementation against oxidative stress have recently been shown in a double-blind, randomized, placebo-controlled trial, with a 247  $\mu\text{g}$  selenium/day supplementation in healthy human volunteers. It improved GSH redox status (47). The presence of selenium as an organic salt in MSBE (0.05%), which accounts for its Recommended Daily Allowance, together with mangiferin, catechin and epicatechin, might be the main properties responsible for the *in vivo* antioxidant mechanism of the whole extract. These antioxidant properties could have been utilized by the cells (especially blood and endothelial cells), sparing the intra- and extracellular antioxidant system and increasing serum peroxyl scavenging capacity, thus preventing age-associated increase in GSH oxidation and lipoperoxidation. It is also possible that MSBE is influencing other cellular systems, suggesting that more detailed examination of other antioxidant parameters is required.

Vimang supplementation may be suggested for its use as a serum protective agent against oxidative damage related to age and also as a prophylaxis as well as adjunct therapy in a variety of age-related diseases involving overproduction of free radicals.

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