Mangifera indica L. EXTRACT (VIMANG) PROTECTS AGAINST LPS INDUCED OXIDATIVE DAMAGE

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Summary

Endotoxic shock causes overproduction of nitric oxide and others oxidant species and different cytokines related with inflammatory processes. Oxidative stress is the major cause of damage associated with elevated NO synthesis, resulting largely from the formation of the peroxinitrite radical. In our laboratory we have investigated the antioxidant and anti-inflammatory properties of an aqueous extract, obtained from the stem bark of *Mangifera indica* L. on different models of oxidative stress and inflammation. In this work we have investigated the effect of endotoxic shock on oxidative damage in rats' serum and the protective effect of a *Mangifera indica* L. extract (Vimang), used in Cuba as a nutritional supplement. Intraperitoneal injection of *E. coli* Lipopolisacharide increased rats' serum TNFα and lipoperoxide. It also diminished total antioxidant status, presuming an oxidative stress. The natural extract and also its most abundant active component, mangiferin, given orally before LPS injection prevented those variations, lowering lipoperoxide measured as Thiobarbituric Reactive Substances and also increased rat serum Total antioxidant Status in a dose dependent manner. Such results show potentiality of *Mangifera indica* L. extract and mangiferin in protecting against oxidative damage originated by excessive or inappropriate production of nitrogen and oxygen reactive substances, TNFα and others pro inflammatory cytokines in different human pathologies.

Keywords: Mangifera indica, Endotoxic shock, Oxidative stress, Antioxidant, Vimang

Introduction

There are considerable evidences that implicate aggressive species of oxygen in the pathogenesis of organ dysfunction consequent to sepsis and septic shock.[1,2] The inflammatory process appears to participate ubiquitously in this setting. A characteristic of inflammation is the involvement of activated neutrophils and their generation of aggressive oxygen species. Such species may both directly injure cells proximal to the oxidant generating cells, and may inactivate any proteolytic mechanisms normally protective against proteolytic injury caused by neutrophil elastase and other proteolytic enzymes released during inflammation. The offending agent in sepsis is most commonly envisioned as bacterial lipopolysaccharide, or endotoxin. Infusion of endotoxin into animals can reproduce much of the pathophysiology of sepsis and septic shock. In such experimental models, it appears that aggressive oxygen species are important actors in the scenario eventuating in cell or organ injury.[3]

Vimang is a standardized aqueous extract of *Mangifera indica* L. stem bark, which has demonstrated potent antioxidant, anti-inflammatory and immunomodulatory properties.[4-8] Its chemical study 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 the xanthone mangiferin, which is the predominant component of this extract (20 %). Vimang is also rich in fatty acids such as myristic, palmitic, estearic, oleic-linoleic and eicosatrienoic.[9]

The present study was undertaken to find out unexplored protective effects of *Mangifera indica* L. extract against oxidative damage and tumor necrosis factor alpha (TNFα) overproduction during endotoxin-induced shock in rats.

Materials and Methods

Drugs

Mangifera indica L extract (Vimang) was kindly donated by The Center of Pharmaceutical Chemistry. Havana. Cuba

Mangiferin (2-β-D-glucopyranosyl-1, 3, 6, 7-tetra-hydroxy-9H-xanthen-9-one) was also supplied by the Laboratory of Analytical Chemistry, Center of Pharmaceutical Chemistry (Cuba). It was purified from *Mangifera indica* L. stem bark standardized extract by extraction with methanol and its purity (95 %) was assessed.[9]

Chemicals

Total antioxidant status kit was purchased from Randox Laboratories Ltd. Bacterial lipopolysaccharide (LPS), thiobarbituric acid, and all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Animals

Female Wistar rats weighing 200–250 g from Centro para la Producción de Animales de Laboratorio (CENPALAB, La Habana, Cuba) were used in these studies. They were kept in a temperature controlled environment (23 ^{o}C) with a 12 h light-dark cycle, relative humidity 40-70 %, with food and water *ad libitum* and fasted overnight (18 h) before the day of the experiments. 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.

Animal models of endotoxic shock

Rats were pre-treated with *Mangifera indica* L. extract (MiE 50, MiE 150, MiE 250) suspended in sterile water (doses: 50, 150, 250 mg/kg) or mangiferin 50 mg/kg (M 50) administered p.o. during seven days before LPS (0.125 mg/kg, i.p.) administration.[10] One hour after LPS arrest, blood of anesthetized rats was extracted by rupture of retro-orbital plexus. Serum was separated by centrifugation at 3603 x g, and frozen at –70 °C until TNFα determination.

TNFα determination

TNFα was measured in serum of rats treated with LPS and supernatants of cell cultures by a standard cytotoxicity assay using L929 line cell in the presence of 1 μg/ml of actinomycin D.[11] Human recombinant TNFα was used as standard (specific activity, 10^7 U/mg).

Lipid peroxidation

The extent of lipid peroxidation was measured by the assay for thiobarbituric acid reactive substances (TBARs) at 535 nm. TBARs were expressed as malondialdehyde equivalents, calculated using an extinction coefficient of 1.56 10^5 M^{-1} cm^{-1}.[12]

Total antioxidant status determination

Antioxidant quantification was carried out in rats' serum using $ABTS^+$ (2,2'-azidodiethylbenzothiazolin sulphanate) radical formation kinetics (Randox Laboratories, Ltd.). The presence of antioxidants in plasma suppresses the bluish-green staining of the $ABTS^+$ cation, which is proportional to the antioxidant concentration level.[13]

Statistical analysis

Statistical significance between independent groups was analyzed by means of non-parametric Kruskal-Wallis tests followed by Mann-Whitney test for paired groups. Pearson correlation analyses were used to find association between variables. P-values less than 0.05 were considered as indicative of significance.

Results and Discussion

TNFα is a major mediator in the pathogenesis of endotoxic shock, and its inhibition has a protective effect in various animal models of sepsis or endotoxin (lipopolysaccharide, LPS) toxicity. *Mangifera indica* L. extract and mangiferin,

administered orally, inhibit the TNFα production during endotoxic shock. The extract inhibited the TNFα serum levels dose-dependently (Figure 1) when it was administered p.o. during seven days previous LPS arrest, with a maximal inhibition of 95.9 % at 250 mg/kg of rats' body weight. Mangiferin (a glucosylxanthone isolated from the extract, 50 mg/kg) also inhibited the TNFα (86.9 %, respect to control groups).

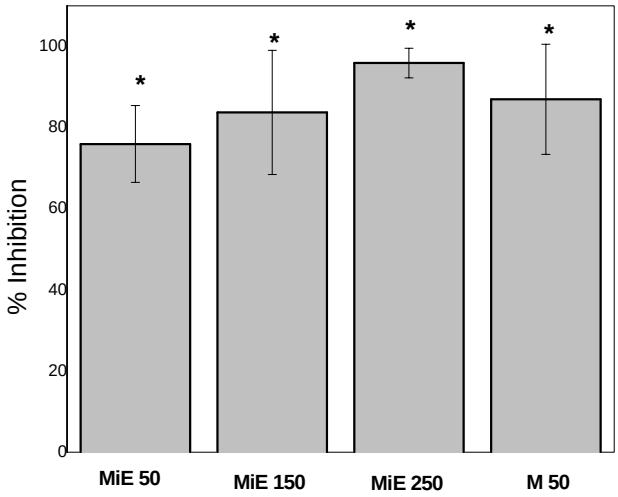

Fig. 1. Rats' serum TNFα inhibition 1 h after intraperitoneal LPS injection by *Mangifera indica* L. extract (MiE) and mangiferin (M). Results are expressed as percent of TNFα inhibition respect to LPS-treated group without any *Mangifera indica* L. extract or mangiferin pre-treatment (Control). Values are means ± S.D. (n= 6). * P< 0.05 respect to Contro

Recently it was demonstrated that Vimang reduced the production of TNFα in macrophage cell line RAW 264.7 stimulated with pro-inflammatory stimuli (IFNγ and LPS).[14)]

Although the molecular bases for the multiple activities assigned to Vimang have not yet been defined, most of the activities inhibited by Vimang require the activation of NF-κB. NF-κB is an ubiquitous transcription factor, which plays a central role in the immune system, by regulating many inflammatory responses through transcriptional activation of certain pro-inflammatory cytokines, the iNOS gene and other genes involved in inflammation.[15)] Recent reports suggest a novel antioxidant which protects against endotoxin-induced shock by inhibiting NF-κB activation.[16)] We have demonstrated that Vimang prevented TNFα-induced IκBα degradation and the binding of NF-κB to the DNA transcriptional activation of NF-κ, which could be the driven mechanism of TNF-α inhibition.[17)]

Figure 2 shows a significant increase of lipoperoxidation measured as Thiobarbituric reactive substance in a control group which received distilled water orally before LPS injection (MiE Free). It was reported that LPS and cytokines such as interleukine-1 and TNFα induced an important inhibition of glutathione peroxidase both in liver during reversible endotoxic shock and in cultured hepatocytes after treatment with endotoxin,

which can justify the lipid peroxidation previously observed.[18] *Mangifera indica* L. extract and mangiferin, given orally before LPS challenge, prevents lipids oxidative damage in a dose-independent manner, through out the reduction of TBARs levels.
This result confirms the antioxidant properties of the extract, which have been proven in others *in vivo* and *in vitro* oxidative stress models.[4-6] Its high content of polyphenolic compounds (gallic acid, 3,4 dihydroxy benzoic acid, benzoic acid, gallic acid methyl ester, gallic acid propyl ester, benzoic acid propyl ester, catechin and epicatechin, the xanthone mangiferin) and its high selenium content could explain such results, mainly because of the antioxidant properties of the former and because selenium is an essential cofactor for glutathione peroxidase, enzyme that maintains the glutathione levels and reduces hydrogen peroxide and organic hydroperoxides.[19] In addition, selenium treatment seems to be promising in severely septic patients.[20]

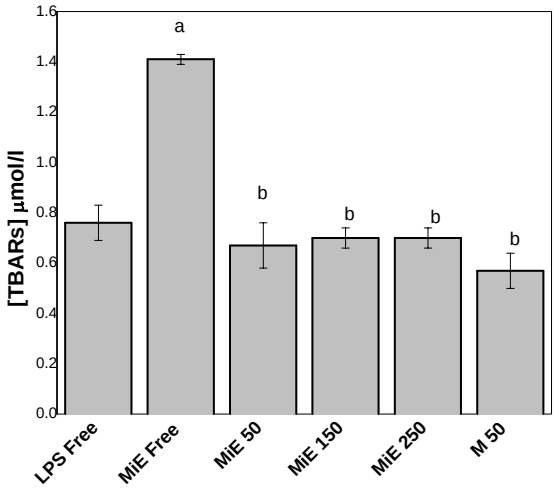

Fig. 2. Influences of *Mangifera indica* L. extract (MiE) and mangiferin (M) pre-treatment on rats' serum TBARs 1 h after intraperitoneal injection of LPS. Experiments were carried out as described in Materials and Methods. Results are expressed as means ± S.D. (n= 6). Groups that not received LPS challenge (LPS Free) and LPS-treated group without any *Mangifera indica* L. extract or mangiferin pre-treatment (MiE Free) were used as controls. [a] $P < 0.05$ compared with LPS Free group. [b] $P < 0.05$ compared with MiE Free group

As can be observed in Figure 3, the natural extract and its major component, mangiferin prevent the reduction of serum antioxidant capacity originated by LPS injection and even increase the antioxidant status of treated groups (MiE 50, MiE 150, MiE 150, M 50) in a dose-dependent manner respect to non-endotoxemic normal groups (LPS Free). Previous report has demonstrated the implication of free radicals in sepsis and septic shock.[1] They have also shown a significant depletion of antioxidants associated with endotoxin challenge.[21-23]

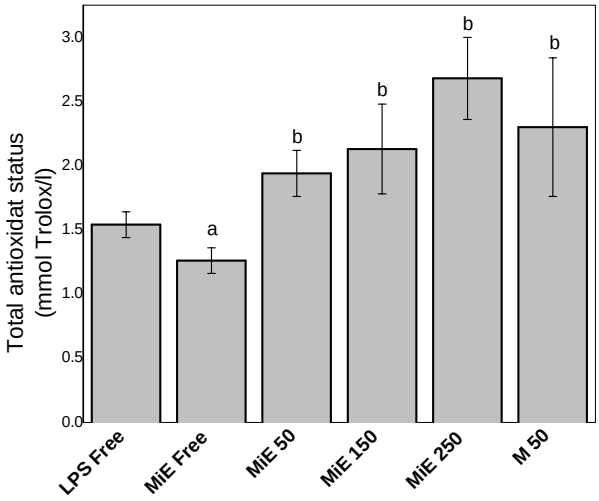

Fig. 3. Influences of *Mangifera indica* L. extract (MiE) and mangiferin (M) pre-treatment on rats' serum Total Antioxidant Status (expressed as Trolox equivalents) 1 h after intraperitoneal injection of LPS. Experiments were carried out as described in Materials and Methods. Results are expressed as means ± S.D. (n= 6). Groups that not received LPS challenge (LPS Free) and LPS-treated group without any *Mangifera indica* L. extract or mangiferin pre-treatment (MiE Free) were used as controls.
[a] $P < 0.05$ compared with LPS Free group. [b] $P < 0.05$ compared with MiE Free group. in Rats Serum 1 h after intraperitoneal LPS injection.

It would be very possible that *Mangifera indica* L. extract and its major polyphenolic component, mangiferin, influence directly serum antioxidant status by their presence in blood, but they could also preserve some other blood antioxidant such as ascorbic acids, α-tocopherol and glutathione.

We found significant negative correlation between TBARs and TAS (Pearson correlation –0.567, $p<0.01$) and TBARs and TNFα inhibition (Pearson correlation –0.667, $p<0.01$) and also a significant positive correlation between TAS and TNFα inhibition (Pearson correlation 0.721, $p<0.01$)

These correlations reflect the possible mechanism by which *Mangifera indica* L. extract protects against oxidative damage and TNFα overproduction associated with endotoxic shock, which appears to be pluripotent, comprising both antioxidative properties and the inhibition of NF-κB. Mangiferin, a glucosylxanthone isolated from the extract, and its most abundant component, is probably the major responsible of these effects. The results suggest that *Mangifera indica* L. extract (Vimang) administration may be a useful adjunct to conventional approaches in the management of septic shock.

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