Original Article

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Neuroprotective and hepatoprotective effects of micronized purified flavonoid fraction (Daflon®) in lipopolysaccharide-treated rats

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ABSTRACT: Micronized purified flavonoid fraction (MPFF, Daflon®) is a phlebotonic drug widely used in chronic venous or lymphatic insufficiency. We aimed to investigate the effects of MPFF on hepatic and brain oxidative stress and on liver injury caused by lipopolysaccharide (LPS) in rats. MPFF (4.5, 9, or 18 mg/kg) or saline was administered orally for two days prior to intraperitoneal (*i.p.*) LPS (300 μg/kg) and at time of LPS administration. Rats were euthanized 4 h after LPS injection. The administration of LPS increased oxidative stress in brain and liver tissue. Malondialdehyde (MDA) increased by 193.5 and 191.8%, reduced glutathione (GSH) decreased by 73.8 and 70.8% and nitric oxide increased by 118.2 and 151.7% in the brain and liver, respectively. Serum paraoxonase 1 (PON1) activity decreased by 42.6%. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were raised by 101.8, 93.6, and 223.2%, respectively. Rats treated with MPFF at 9 and 18 mg/kg showed decreased brain MDA (27.5-34%), nitrite (25.5-41%) and increased GSH (27.2-74.1%). In the liver, MDA decreased by 16.4-59.8%, nitrite decreased by 54.7-56.7%, and GSH increased by 15.2-70.5% with MPFF at 4.5, 9, or 18 mg/kg, respectively. Serum PON1 activity showed 41-65.9% increments with MPFF. Significant reductions in serum AST, ALT, and ALP were seen after treatment with MPFF. Moreover, the degree of histological damage, expression of the inducible form of nitric oxide synthase and the apoptotic enzyme caspase-3 in the liver were substantially reduced. MPFF thus prevented the increased oxidative stress and inflammation in brain and liver as well as the liver dysfunction caused by endotoxemia in the rat.

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1. Introduction

Micronized purified flavonoid fraction (MPFF, Daflon®) is a semisynthetic drug which consists of 90% micronized diosmin (a flavone derivative) and 10% flavonoids expressed as hesperidin (a flavonone derivative). The flavonoid glycosides diosmin and hesperidin occur naturally in citrus fruit (*1*). The drug is widely used in treatment of varicose veins and venous ulcers, lymphatic insufficiency and hemorrhoids (*2,3*). In these conditions, MPFF exerts a venotonic action, decreasing venous reflux, and thereby alleviating edema and providing effective venous drainage (*4*). Moreover, the drug has been shown to provide better outcomes for patients with impaired cardiac function before undergoing cardiac operations that require cardiopulmonary bypass (*5*). These effects of MPFF can be ascribed to the anti-inflammatory, microcirculatory, and antioxidant effects of its flavonoid substances. In this context, MPFF has been shown to decrease the levels of granulocyte and macrophage infiltration into the inflamed tissues as well as leucocyte adhesion to the vascular endothelium. The decrease in release of oxygen free radicals, cytokines, and proteolytic matrix metalloproteinases from activated inflammatory and endothelial cells, results in lower levels of inflammation, decreased microvascular permeability and decreased leukocyte-dependent endothelial damage (*6,7*). MPFF decreases vascular permeability more than any of its single constituents, suggesting that the flavonoids present in its formulation have a synergistic action (*8*). The drug possesses an antioxidant effect, significantly decreasing the level of hydroxyl free radicals (*9*), increasing free SH-group concentration, and natural scavenger capacity (*10*).

Lipopolysaccharide (LPS)-induced endotoxemia is a well-established model for infection with Gram-

negative bacteria. By acting on Toll-like receptor 4 (TLR4) on immune cells such as monocytes, macrophages, neutrophils and dendritic cells, LPS triggers synthesis and release of proinflammatory cytokines and nitric oxide both in the periphery and central nervous system, resulting in peripheral and neuroinflammation (*11,12*). Since neuroinflammation and oxidative stress are important contributors to the pathogenesis and disease progression of some neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease (*13,14*), LPS-endotoxemia represents a useful model for studying the effect of systemic inflammation on brain function (*15*).

The present study was therefore designed to investigate the effects of MPFF on oxidative stress in brain and liver of rats subjected to endotoxemia and systemic inflammation caused by *Escherichia coli* LPS injection. In addition, this study aimed to investigate whether treatment of LPS-rats with MPFF would protect against endotoxemic liver injury.

2. Materials and Methods

2.1. *Animals*

Sprague-Dawley rats of both sexes, weighing 120-130 g were used throughout the experiments and fed with standard laboratory chow and water *ad libitum*. All animal procedures were performed in accordance with the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985).

2.2. *Drugs and chemicals*

A purified lyophilized *E. coli* endotoxin (Serotype 055:B5, Sigma, St Louis, MO, USA) was used and dissolved in sterile saline, aliquoted, and frozen at −20°C. MPFF, (Daflon®, Servier, Paris, France) consisting of 90% diosmin and 10% hesperidin, was dissolved in isotonic (0.9% NaCl) saline solution immediately before use. The doses of MPFF were based upon the human dose after conversion to that of the rat according to Paget and Barnes (*16*) conversion tables.

2.3. *Study design*

Rats were randomly divided into 5 equal groups (6 rats each). Rats were treated with vehicle (group 1) or MPFF (4.5, 9, or 18 mg/kg) once daily orally for 2 days prior to and at the time of endotoxin administration (LPS: 300 µg/kg, *i.p.*). The fifth group (n = 6) received only the vehicle (control). Four hours after LPS or vehicle injection, blood samples were obtained from the retro-orbital venous plexus under ether anesthesia. Rats were then euthanized by decapitation under ether anesthesia, livers and brains were then removed, and washed with ice-cold phosphate buffered saline (PBS, pH7.4), and parts of the tissues were preserved in formalin 10% for further histopathological and immunohistochemical examination. Other parts were weighed and stored at −80ºC for biochemical analyses. The tissues were homogenized with 0.1 M phosphate buffered saline at pH 7.4, to give a final concentration of 10% (w/v) for the biochemical assays. The time selected for tissue sampling (4 h after *i.p.* administration of LPS) was based on previous studies that indicated the rise in plasma and tissue cytokines and inflammatory mediators (interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS) mRNA expression, nitric oxide, and myeloperoxidase activity) in rats receiving *i.p.* LPS by that time (*17,18*).

2.4. *Determination of lipid peroxidation*

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the tissue homogenates. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea *et al.* (*19*), in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having a peak absorbance at 532 nm (UV-VI8 Recording Spectrophotometer, Shimadzu, Kyto, Japan).

2.5. *Determination of reduced glutathione*

Reduced glutathione (GSH) was determined in tissue by Ellman's method (*20*). The procedure is based on the reduction of Ellman's reagent by –SH groups of GSH to form 2-nitro-5-mercaptobenzoic acid, the nitromercaptobenzoic acid anion has an intense yellow color which can be determined spectrophotometrically.

2.6. *Determination of nitric oxide*

Nitric oxide measured as nitrite was determined by using Griess reagent, according to the method of Moshage *et al.* (*21*), where nitrite, stable end product of the nitric oxide radical, is mostly used as an indicator for the production of nitric oxide.

2.7. *Determination of paraoxonase activity*

Arylesterase activity of paraoxonase was measured spectrophotometrically in serum following the procedure described by Higashino *et al*. (*22*) and Watson *et al*. (*23*) using phenyl acetate (Sigma) as substrate.

2.8. *Determination of serum liver enzymes*

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes, indicators of liver damage, were measured in serum according to the Reitman-Frankel colorimetric transaminase procedure (*24*), whereas colorimetric determination of

alkaline phosphatase (ALP) activity was done according to the method of Belfield and Goldberg (*25*), using commercially available kits (BioMérieux, France).

2.9. *Histological assessment of liver injury*

Liver sections from each rat were fixed in freshly prepared 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Five µm thick paraffin sections were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination. Sections were examined using a light microscope.

2.10. *Immunohistochemical assessment of liver injury*

Immunohistochemical staining of anti-caspase-3 antibody and iNOS was performed with streptavidin-biotin. Sections of four µm thick were deparaffinized and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min at room temperature. The specimens were then incubated with anti-caspase-3 and iNOS antibody as the primer antibody at a 1:100 dilution. The specimens were counterstained with H&E. Negative controls were prepared by substituting normal mouse serum for each primary antibody.

2.11. *Statistical analysis*

Data are expressed as mean ± SE. Statistical analysis of the data was done using one way ANOVA followed by the Duncan test for multiple group comparison tests, using SPSS software (SAS Institute Inc., Cary, NC, USA). Probability levels of $p < 0.05$ were considered statistically significant.

3. Results

3.1. *Biochemical results*

3.1.1. *Effect of MPFF on brain oxidative stress*

The administration of LPS significantly increased brain MDA by 193.4% (91.6 ± 3.4 *vs.* 31.2 ± 2.2 nmol/g, $p < 0.05$). GSH decreased by 73.8% (0.896 ± 0.03 *vs.* 3.42 ± 0.18 µmol/g, $p < 0.05$), while nitric oxide (the level of nitrite) increased by 118.2% (48.0 ± 2.7 *vs.* 22.0 ± 1.0 µmol/g, $p < 0.05$) after LPS injection compared with the saline control group. Brain MDA was significantly decreased by 21.7, 27.5, and 34% after MPFF at 4.5, 9, or 18 mg/kg, respectively (71.7 ± 3.1, 66.4 ± 4.2, and 60.4 ± 3.9 *vs*. 91.6 ± 3.4 nmol/g, $p < 0.05$) (Figure 1A). The administration of MPFF at 9 and 18 mg/kg resulted in a 27.2 and 73.1% increase in GSH (1.14 ± 0.08 and 1.56 ± 0.06 *vs*. 0.896 ± 0.03 µmol/g, $p < 0.05$) (Figure 1B). The level of nitric oxide decreased by 25.5 and 41.0% after MPFF at 9 and18 mg/kg (38.1 ± 2.1 and 28.3 ± 1.4 *vs*. 48.0 ± 2.7 µmol/g, $p < 0.05$) (Figure 1C).

3.1.2. *Effect of MPFF on liver oxidative stress*

Liver MDA was increased significantly by 191.8% following endotoxin injection (151.2 ± 6.4 *vs.* 51.8 ± 2.5 nmol/g, $p < 0.05$). A significant decrease in GSH by 70.8% (1.05 ± 0.06 *vs*. 3.6 ± 0.18 µmol/g, $p < 0.05$) as well as markedly raised nitric oxide (40.3 ± 2.2 *vs*. 16.0 ± 1.3 µmol/g, $p < 0.05$) were observed after LPS treatment. The administration of MPFF at 9 and 18 mg/kg resulted in a significant decrease in liver MDA by 16.4 and 59.7% compared to the LPS control group (122.1 ± 4.1 and 60.9 ± 3.8 *vs*. 151.2 ± 6.4 nmol/g, $p < 0.05$) (Figure 2A). There was a dose-dependent increase in liver GSH by 15.2, 43.8,

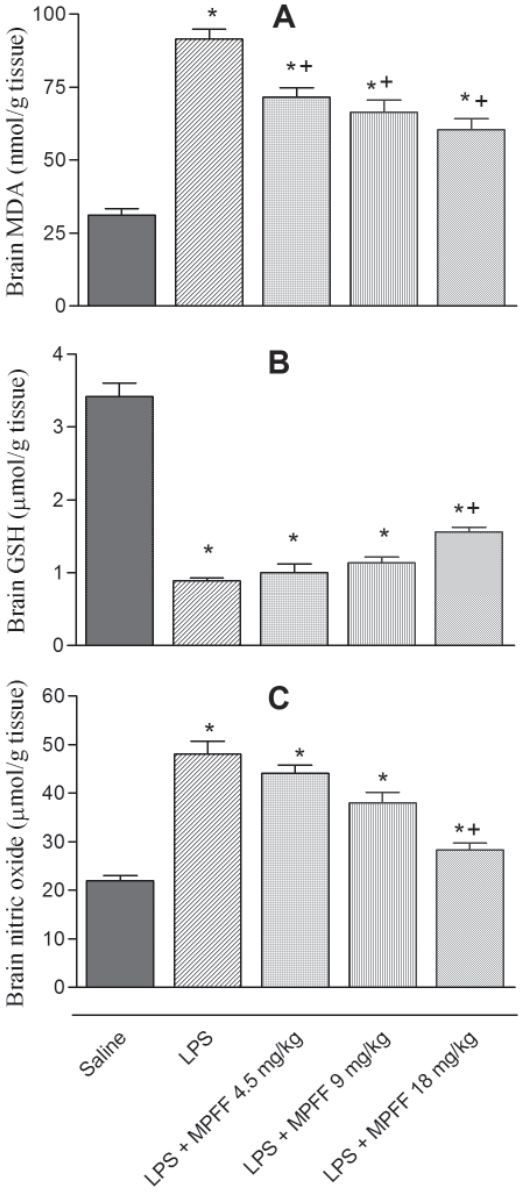

Figure 1. Effect of MPFF on LPS-induced changes in brain MDA (A), GSH (B), and nitric oxide (C). Data are expressed as mean ± SE, $n = 6$. * $p < 0.05$ *versus* saline control; + $p < 0.05$ *versus* LPS (one-way analysis of variance and Duncan multiple range test).

and 70.5% (1.21 ± 0.03, 1.51 ± 0.08, and 1.79 ± 0.06 *vs*. 1.05 ± 0.06 µmol/g, $p < 0.05$) as well as a dose-dependent decrease in nitrite by 54.7, 55.3, and 56.7% (18.2 ± 1.0, 18.0 ± 1.2, and 16.5 ± 0.9 *vs*. 40.3 ± 2.2 µmol/g, $p < 0.05$) after treatment with MPFF at 4.5, 9, or 18 mg/kg, respectively (Figure 2C).

3.1.3. *Effect of MPFF on serum liver enzymes*

In rats treated with only LPS, the levels of ALT, AST, and ALP in plasma were markedly raised by 93.6% (36.6 ± 2.0 *vs*. 18.9 ± 1.2 U/L), 101.8% (123.1 ± 7.2 *vs*. 61.0 ± 3.4 U/L) and 223.2% (446.0 ± 12.8 *vs*. 138 ± 6.9 U/L), respectively. Significant reduction in serum AST, ALT, and ALP were observed in rats treated with MPFF. Thus, ALT decreased by 17.2, 33.9, and 45.3% after treatment with MPFF at 4.5, 9, or 18 mg/kg, respectively; AST decreased by 17 and 20% by MPFF at 9 or 18 mg/kg, respectively; ALP decreased by 29.1, 37.8, and 44% by MPFF at 4.5, 9, or 18 mg/kg, respectively (Figures 3).

3.1.4. *Effect of MPFF on serum paraoxonase 1 (PON1) activity*

Serum PON1 activity decreased by 42.6% following endotoxin administration (83.8 ± 4.6 *vs*. 146.1 ± 7.8 kU/L, $p < 0.05$). The administration of MPFF resulted in a significant and a dose-related increase in PON1

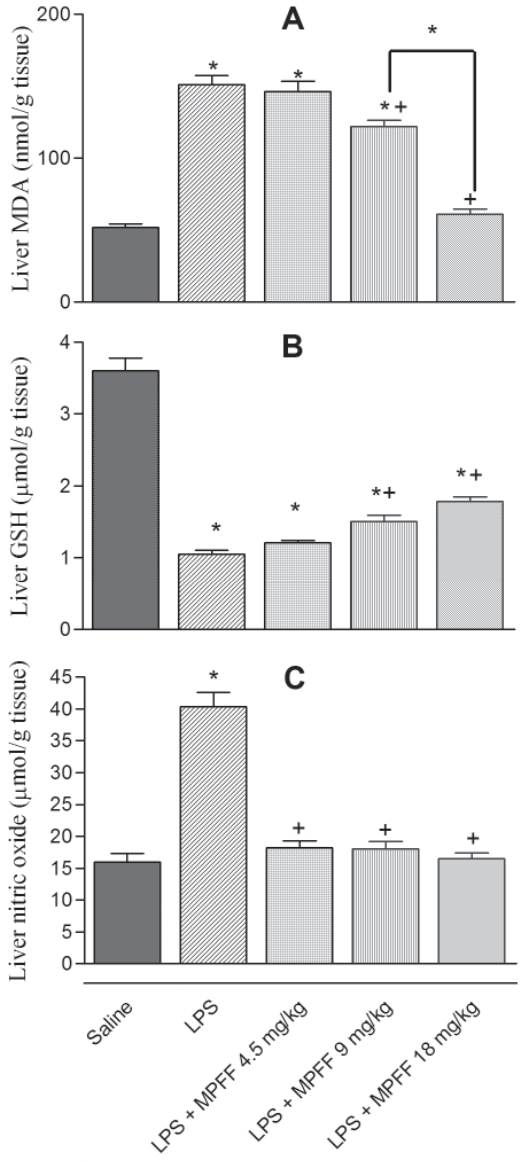

Figure 2. Effect of MPFF on LPS-induced changes in liver MDA (A), GSH (B), and nitric oxide (C). Data are expressed as mean ± SE, $n = 6$. * $p < 0.05$ *versus* saline control and between different groups as indicated in the figure; + $p < 0.05$ *versus* LPS (one-way analysis of variance and Duncan multiple range test).

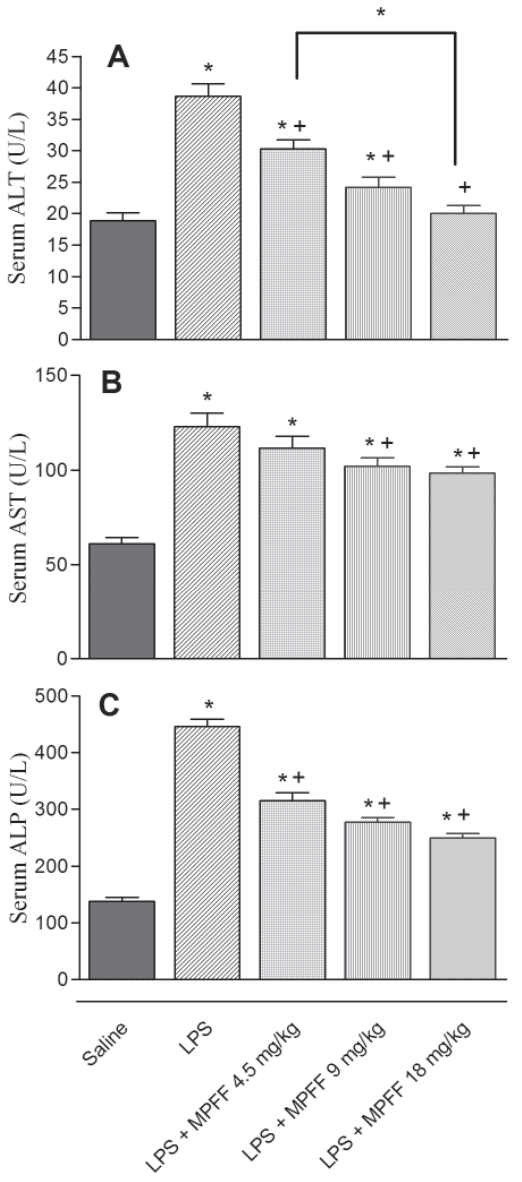

Figure 3. Effect of MPFF on LPS-induced elevation in serum ALT (A); AST (B), and ALP (C). Data are expressed as mean ± SE, $n = 6$. * $p < 0.05$ *versus* saline control and between different groups as indicated in the figure; + $p < 0.05$ *versus* LPS (one-way analysis of variance and Duncan multiple range test).

activity in serum by 41, 56.7, and 65.9% (118.2 ± 6.0, 131.3 ± 5.1, and 139.1 ± 4.8 *vs*. 83.8 ± 4.8 kU/L, $p < 0.05$) after treatment with MPFF at 4.5, 9, or 18 mg/kg, respectively (Figure 4).

3.2. *Histopathological results*

The liver of the control (saline-treated) rats showed normal hepatic architecture with distinct hepatic cells, sinusoidal spaces and a central vein (Figure 5A). Examination of liver sections from LPS-treated rats revealed inflammatory leukocytic cell infiltration around the central vein, and hydropic degeneration with pykontic nuclei (Figure 5B). Focal necrotic areas with inflammatory cell reaction, sinusoidal dilation and activated Kupffer cells were seen (Figure 5C). The administration of MPFF resulted in a significant decrease in liver inflammation and necrosis compared to the LPS control group. The effect was dose-dependent. Thus, liver sections of rats treated with LPS and MPFF at 4.5 mg/kg showed apparently normal tissues with congestion of the central vein and dilation of sinusoids. Minimal focal necrotic areas were also visible (Figure 5D). After treatment with MPFF at 9 mg/kg, sections revealed apparently normal tissues with a mildly congested central hepatic vein and some of the sinusoids. Focal necrotic areas were not seen (Figure 5E). Liver sections of rats treated with LPS and MPFF at 18 mg/kg showed almost normal liver with very mild dilation of sinusoids and no congestion. The nuclei were normal indicating the recovery of the liver tissues (Figure 5F).

3.3. *Immunohistochemical results*

3.3.1. *Caspase-3 expression*

Expression of caspase-3 was not observed in control liver (Figure 6A). By comparison, strong expression of caspase-3 was observed in the LPS control group as shown in Figure 6B and gradually decreased in rats treated with MPFF in a dose-dependent manner as shown in Figures 6C-6E.

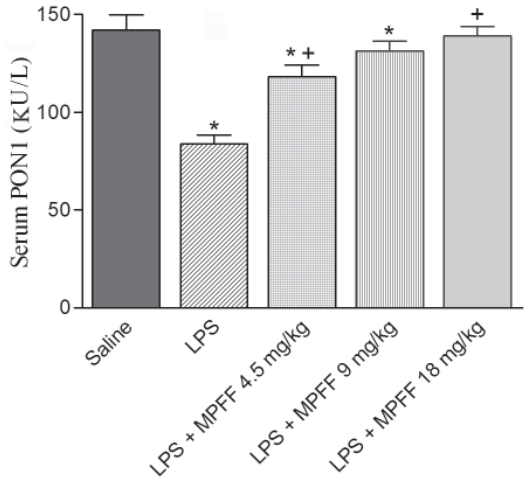

Figure 4. Effect of MPFF on serum PON1 activity in LPS-treated rats. Data are expressed as mean ± SE, $n = 6$.* $p < 0.05$ *versus* saline control; + $p < 0.05$ *versus* LPS (one-way analysis of variance and Duncan multiple range test).

3.3.2. *iNOS expression*

In the liver tissue of control rats there was a weakly localized iNOS immunohistochemical staining (Figure 7A). In rats treated with LPS, a much more intense expression of iNOS was detected in the hepatocytes of the centrilobular zone in the surface of hepatocytes. A number of hepatocyte nuclei showed iNOS immunoreaction (Figure 7B). In hepatocytes of rats treated with LPS and MPFF, iNOS immunoreactivity showed a dose-dependent decrease compared with the LPS control group (Figures 7C-7E).

4. Discussion

The results of the present study indicate that pretreatment with MPFF was able to ameliorate brain

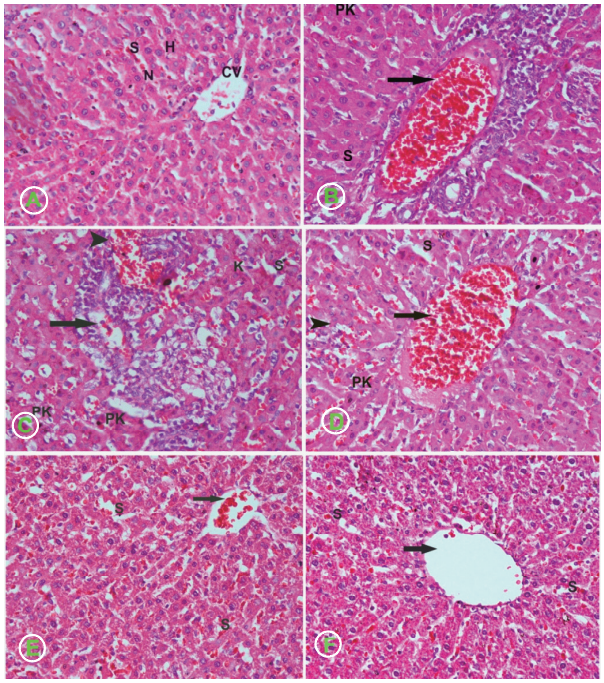

Figure 5. H&E stained liver sections from: (A) control (saline-treated) rat showing central vein (CV), hepatic cells (H), sinusoidal space (S), and nucleus (N); **(B)** LPS-treated rat showing inflammatory leukocytic cell infiltration around central vein (arrow), sinusoidal dilation (S), and pyknotic nuclei (PK); **(C)** LPS-treated rat showing focal necrotic area (arrow) with inflammatory cell reaction (arrow head), congestion and sinusoidal dilation (S), activated Kupffer cell (K) and pyknotic cells; **(D)** LPS + MPFF 4.5 mg/kg-treated rat showing severly congested hepatic central vein and focal area of mild mononuclear cells; **(E)** LPS + MPFF 9 mg/kg-treated rat showing moderately normal tissue with mildly congested central hepatic vein and some of the sinusoids. Focal necrotic areas were not visible; **(F)** LPS + MPFF 18 mg/kg-treated rat showing almost normal liver with very mild dilation of sinusoids and no congestion. The nuclei were normal indicating the recovery of the liver tissues (H&E, ×400).

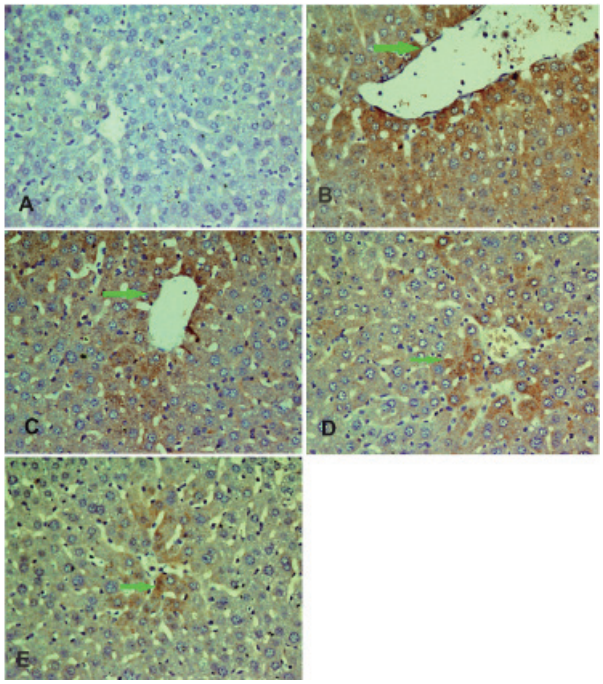

Figure 6. Effect of MPFF on LPS-induced caspase-3 expression in liver: caspase-3 immunohistochemistry of liver from a rat treated with **(A)** saline (control): caspase-3-immunolabeled cells were rarely present; **(B)** only LPS: an increased number of caspase-3 immunolabeled hepatocytes were observed around central veins compared to control animals, suggesting increased apoptosis; **(C)** LPS + MPFF 4.5 mg/kg: caspase-3-immunolabeled cells were slightly decreased compared to LPS control group; **(D)** LPS + MPFF 9 mg/kg: caspase-3-immunolabeled cells were slightly decreased compared to LPS control group; **(E)** LPS + MPFF 18 mg/kg: caspase-3-immunolabeled cells were obviously decreased compared to LPS control group (caspase-3 immune staining, ×400).

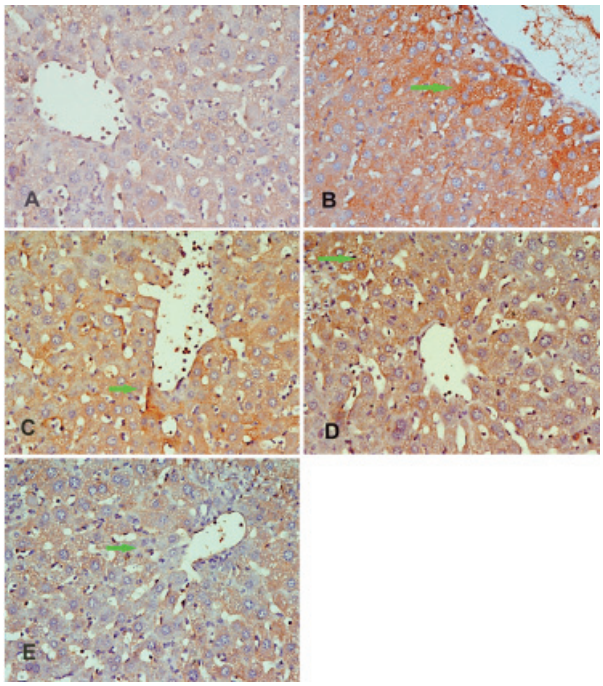

Figure 7. Effect of MPFF on LPS-induced iNOS protein expression in liver: iNOS immunohistochemistry of liver from a rat treated with **(A)** saline (control): iNOS-immunolabeled cells were weakly present in the liver of control rats; **(B)** only LPS: a marked increased number of iNOS immunolabeled hepatocytes was observed around central veins compared to normal animals; **(C)** LPS + MPFF 4.5 mg/kg: iNOS immunolabeled cells were slightly decreased compared to LPS control group; **(D)** LPS + MPFF 9 mg/kg: iNOS immunolabeled cells were slightly decreased compared to LPS control group; **(E)** LPS + MPFF 18 mg/kg: iNOS immunolabeled cells were markedly decreased compared to LPS control group (iNOS immune staining, ×400).

and liver oxidative stress induced by the intraperitoneal administration of LPS. The drug lessened the elevation in MDA, a marker of increased oxidative stress, which indicates a free radical attack on polyunsaturated fatty acids of biological membranes (*26*). The increase in nitric oxide in response to LPS was also decreased by treatment with MPFF. Nitric oxide generated by the inducible form of nitric oxide synthase (iNOS) is most often associated with inflammatory conditions in which it is produced in large amounts by monocyte/macrophage lineage cell types. The induction of NOS has been demonstrated in response to a number of stimuli including LPS, IL-1, and TNF-α (*27*). Glutathione is an intracellular tripeptide (γ-glutamyl-cysteinyl-glycine) common in all tissues and is the most important thiol antioxidant in the cell (*28*). The administration of LPS endotoxin was associated with decreased levels of GSH in the brain and liver. This decline in GSH decreased following treatment with MPFF. Collectively, these data suggest a beneficial effect for MPFF during systemic inflammatory illness.

Studies have indicated that the brain is affected during systemic inflammation. Thus, peripheral inflammation induced by intraperitoneal LPS injection produces brain inflammation and oxidative injury (*15*). This is also evident in the present study which shows increased brain MDA and nitrite levels after LPS. Inflammatory cytokines *e.g.*, IL-1, IL-6, and TNF-α secreted by peripheral innate immune cells during endotoxemia, use neural (*29*) and blood brain barrier pathways (*30*) to relay inflammatory signals to the brain resulting in activation of macrophages and microglia to produce cytokines and free radicals. Such events can induce neuronal dysfunction/degeneration (*15,31*). Studies also suggested that peripheral inflammatory stimuli can aggravate underlying brain pathology *e.g.*, exacerbate brain ischemic injury (*31*) and facilitate microtubule-associated protein (tau) phosphorylation, one of the key pathologies in the brain of patients with Alzheimer's disease (*32*). There is also ample evidence suggesting that increased levels of oxidative stress in brain is linked with aging (*33*) and with development of several neurodegenerative diseases *e.g.*, Parkinson's disease, Alzheimer's disease (*13*), and multiple sclerosis (*34*) as well as in psychiatric diseases *e.g.*, schizophrenia (*35*). Drugs that cause reduction in oxidative damage therefore represent an important therapeutic strategy to slow or halt these disease processes and hence, emphasize the importance of the findings of the present study.

In the present study, the administration of LPS was associated with liver damage. A significant rise in serum

hepatocellular enzymes ALT and AST as well as of the cell wall enzyme ALP was observed. Histologically, focal necrotic areas, inflammatory cell infiltration and hydropic degeneration were seen. Pretreatment of LPS-rats with MPFF significantly attenuated this liver dysfunction. The release of liver enzymes into the circulation was decreased by the drug in a dose-dependent manner and the histological degree of hepatic injury due to endotoxemia was markedly improved by pretreatment with MPFF. Studies have indicated increased iNOS mRNA expression in several organs (*18,36,37*) 4 h after *i.p.* administration of LPS. In the present study, iNOS inmmunoreactivity in the liver increased after LPS where an intense expression of iNOS was detected in the hepatocytes of the centrilobular zone in the surface of hepatocytes. iNOS immunolabeled cells were markedly decreased by the higher dose of MPFF. Caspases are involved in the process of apoptosis or programmed cell death. Caspase-3 is a frequently activated death protease, which disassembles the cell by catalyzing the specific cleavage of many key cellular proteins leading to rapid cell death (*38*). In the present study apoptosis was assessed in liver sections using antibodies that specifically recognize activated caspase-3 (*39*). Increased immunoreactivity of caspase-3 was observed in the cytoplasm of the hepatocytes following LPS challenge. This decreased after pretreatment with MPFF, thereby, indicating decreased apoptosis by the drug. These data clearly indicate hepatic protective effects for MPFF against the deleterious effects of systemic endotoxemia.

The present study also showed that pretreatment with MPFF protected against the decline in serum PON1 induced by endotoxemia. PON1 is a calcium-dependent serum esterase that is synthesized by the liver and released into the circulation, where it binds mainly to high-density lipoproteins and is thought to play an important role in the protection of low-density lipoprotein against oxidative modification (*23*). PON1 also plays an important role in the metabolism of many xenobiotic compounds (*40*). The enzyme is likely to serve an antioxidant function and PON1 activity has been shown to be decreased in several pathologic states such as rheumatoid arthritis (*41*), coronary heart disease (*42*), chronic hepatitis, liver cirrhosis (*43*), and multiple sclerosis in relapse (*44*). In the present study serum PON1 activity decreased following endotoxin administration. In their study, Feingold *et al*. (*45*) observed decreased serum PON1 activity within 24 h following LPS treatment and at doses as low as 100 ng/kg. LPS also induced a marked decrease in PON1 mRNA in the liver as early as 4 h after a single LPS treatment. Paraoxonase might also represent an early defense mechanism against elevated levels of oxidative stress (*46*). In the present study, the administration of MPFF was associated with a dose-dependent increase of PON1 activity in the serum of LPS-treated rats. This PON1 response is likely to reflect reduction of oxidative stress by MPFF with sparing of the enzyme.

MPFF is a vasotonic drug that is widely used to improve disorders of venous or lymphatic origin (*2,3*). The drug is safe with no or minor side effects being reported (*47*). MPFF owes its beneficial effects to the ability of its content of different flavonoids to decrease leukocytic infiltration and adhesion to the vascular endothelium, resulting in reduced levels of proteolytic enzymes, and decreased microvascular permeability (*7*). The drug possesses antioxidant effects as well (*9,10*). The present study shows that the administration of MPFF is associated with hepatic protective effects. The present study is also the first to demonstrate the inhibitory effect of MPFF pretreatment on the brain oxidative stress in an *in vivo* model of systemic inflammation induced by LPS endotoxin. These findings derive their significance from the evidence that oxidative stress and neuroinflammation are important contributors in the pathogenesis of several neurodegenerative disorders. Oxidative stress also contributes to age-associated neurodegeneration. Orally administered MPFF, therefore, might be a useful adjunct in the treatment of these disorders.

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