

Protective Activity of *Markhamia tomentosa* (Benth.) K. Schum. (Bignoniaceae) Methanol Leaves Extract against D-Galactosamine/Lipopolysaccharide-Induced Acute Liver Injury in Mice

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Abstract

Markhamia tomentosa (Benth.) K. Schum. (*Mt*) is a Cameroonian medicinal plant, traditionally used to treat painful and inflammatory illness. This study aimed to examine the effects of methanol leaves extract (MLE) of *Mt* in D-galactosamine (D-GaIN)/lipopolysaccharide (LPS)-induced liver injury. The MLE (100 and 200 mg/kg), Ascorbic acid (10 mg/kg) and distilled water were administered 12 h and 1 h before intraperitoneal injection of D-GaIN (10 mg/mouse)/LPS (0.1 µg/g). Animals were sacrificed 6 h after D-GaIN/LPS challenge. Liver injury was assessed biochemically by determination of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), superoxide dismutase (SOD) and catalase activities. Malondialdehyde (MDA), reduced glutathione (GSH), nitrites, total protein and bilirubin levels were explored. Histopathological examination of liver tissue was also performed. Liver enzymes (ALAT, ASAT, ALP) activity, nitrites, MDA and bilirubin levels were increased, while protein level, SOD and catalase activities were significantly reduced by D-GaIN/LPS administration. MLE (100 or 200 mg/kg) protected mice against D-GaIN/LPS-induced death. In addition, the plant extract significantly reduced ALAT and ALP activity, exhibiting 23.00% and 62.20% protection, respectively. SOD activity and total protein were significantly ($p < 0.05$) increased by the plant extract. Total bilirubin and MDA levels were reduced ($p < 0.01$) by 37.75% and 62.79%, respectively in animal treated with MLE. Histological analysis of liver sections showed

that MLE (100 or 200 mg/kg) protected mice against D-GalN/LPS-induced liver injury. The obtained results showed that MLE of *Mt* may possess hepatoprotective effects. Protection afforded by MLE against D-GalN/LPS-induced fulminant liver injury may result from reduction of oxidative stress.

Keywords

Markhamia tomentosa, D-Galactosamine/Lipopolysaccharide, Oxidative Stress, Liver Injury

1. Introduction

There is an increasing interest in phytotherapy across the world [1]. Medicinal plants have been explored over years and some of currently available drugs are from plants' origin [2]. World Health Organization has pointed out the importance of research to support the development of traditional herbal medicine in delivering appropriate, safe and effective treatments [3].

About 800 species of trees, shrubs or lianas exist in the Bignoniaceae Juss. family [4]. They are largely found in tropics and few species are distributed in the temperate and sub-tropical regions [5]. They are mostly used for medicinal and ornamental purposes. *Markhamia tomentosa*, a tree of about 15 m high, found in the savannah forests, throughout West Africa and extending southward to Angola belongs to this family. *M. tomentosa* carries large yellow flowers in long terminal racemes and is quite decorative when flowering. Leaves are opposite with simple pinnately compound. In Cameroon, this plant is commonly known as “bougton” in Bayangam, “bobèdou” in Duala, “abbe” in Koosi or “mawelu” in Kpe [6].

The stem bark of *M. tomentosa* is used against chest pain while the leaf is used to cure headache, lumbago, oedema and gout [7]. Leaves and barks preparations are administered as rejuvenating, diuretic medicine for legs oedema and elephantiasis of the scrotum. These preparations are also used to treat canker, rheumatic pain, diseases of the respiratory tract and bouts of swamp-fever, constipation and fever [8] [9]. Ethnopharmacological data indicate some claimed therapeutic uses of this plant against snake bite/venom, sore eyes, heart pain [10], general pains, headache or backache [11]. *M. tomentosa* leaves are reported to cure diseases related to the reproductive system [12].

Previous studies showed that ethyl acetate, dichloromethane and methanol leaves extracts of *M. tomentosa* exhibited antioxidant and antimicrobial activities [10]. Phytochemical investigations revealed that saponins, tannins, anthraquinones, alkaloids, glycosides, cardiac glycosides, flavonoids and phenols are found in *M. tomentosa* methanol extract [13]. Eight compounds were identified in the ethyl acetate extract among which, 2-acetylnaphtho[2,3-b]furan-4,9-dione and 2-acetyl-6-methoxynaphtho[2,3-b]furan-4,9-dione exhibited potent anti-protozoal activity and high toxicity on myeloblast of rat skeletal muscle [14]. It

was demonstrated that *M. tomentosa* leaf extracts possessed analgesic effects and the most potent was recorded with methanol leaf extract. This extract was able to inhibit carrageenan-induced paw oedema [13]. Anti-inflammatory activities of methanol leaves extract (MLE) were investigated on xylene, histamine and serotonin-induced acute inflammation, and on formalin and cotton pellet-induced chronic inflammation [15]. Anti-arthritic effects of methanol leaf extract of *M. tomentosa* on complete Freund's adjuvant induced arthritis were proved [16]. However, from the best of our knowledge, there is apparently no study available on MLE effects against experimental liver injury. This study was therefore undertaken to examine the hepatoprotective effects of MLE against acute D-GalN/LPS-induced fulminant liver injury in mice.

2. Materials and Methods

2.1. Plant Material

The leaves of *Markhamia tomentosa* were harvested in November 2009 in Yaounde, Cameroon. The plant was identified and authenticated by botanists of the Cameroon National Herbarium, by comparison to an existing voucher specimen registered under number 1974/SRFK.

2.2. Method for Preparation of Plant Extract

The air-dried leaves were pulverized in a motor-driven grinder and used to prepare extracts. A bioassay-guided extraction approach was used to determine the biological activities of different extracts. A sequential extraction was done based on the protocol described by Sosa *et al.* [17] with little modification. Five hundred grams of leaves powder of *M. tomentosa* were successively macerated for 72 h in hexane (1000 mL), dichloromethane (1000 mL), ethyl acetate (1000 mL) and methanol (1000 mL), and for 24 h in boiling distilled water (1000 mL). The macerate was air-dried and weighed after each extraction. Each filtrate was dried under reduced pressure, except water filtrate which was evaporated in an oven at 45°C. The yields of hexane, ethyl acetate, dichloromethane, methanol and aqueous extracts were 0.11%, 1.40%, 1.31%, 6.30% and 5.30%, respectively. Based on previous study [13], the methanol extract which was the most active extract was used for this experimentation and orally administered to animals.

2.3. Chemicals

Chemical substances used in the study were D-galactosamine hydrochloride (Sigma, USA), lipopolysaccharide (Sigma, USA), ascorbic acid (Fourrts, Chennai); Trichloroacetic acid, thiobarbituric acid, sulphanilamide, naphthylethylenediamide, phosphoric acid and DTNB (Sigma Aldrich, Germany); ALT, AST and bilirubin kits (Fortress Diagnostics Limited, UK) and ALP kits (Chronolab Systems, Spain).

2.4. Animals

Eight to ten weeks old male Swiss albino mice (20 - 25 g) used for this study

were bred in the animal house of the Department of Animal Biology and Physiology, University of Yaounde I, Cameroon. The animals were grouped and housed in plastic cages ($\text{Ø}40 \times 15$ cm) with no more than five animals per cage. These animals were kept under natural conditions (ambient temperature with 12:12 h light/dark cycle) and were allowed free access to standard commercial diet with water *ad libitum*. Animals were used and cared in agreement with internationally standard guidelines for animal use. Ethical clearance for using animals in the present study, was obtained from the Cameroon National Ethical Committee (Reg. N° FWAIRD 0001954). Animals were fasted 12 h before the experiment.

2.5. Experimental Design

Thirty mice were randomly divided into five groups of 6 animals each, including normal saline + distilled water group, D-GalN/LPS + distilled water group, D-GalN/LPS + MLE (100 or 200 mg/kg) groups and D-GalN/LPS + ascorbic acid (10 mg/kg) group. *M. tomentosa* methanol extract at the dose of 100 or 200 mg/kg and ascorbic acid at the dose of 10 mg/kg were orally administered to D-GalN/LPS + MLE groups and D-GalN/LPS + ascorbic acid group, respectively, at 12 h and 1 h before induction of hepatitis. Normal saline and D-GalN/LPS groups were given only distilled water (*p. o.*). One hour after the treatment of mice, all animals except normal saline group received intraperitoneal injection of D-galactosamine hydrochloride (10 mg/mouse) and lipopolysaccharide (0.1 $\mu\text{g/g}$ of body weight). The animals were sacrificed by decapitation 6 hours after D-GalN/LPS injection. Blood samples were collected in test tube and were used for serum preparation. The liver of each mice was removed, rinsed in normal saline solution. Two samples of the liver tissue were collected, one of them was homogenized (20% homogenate) in cold Tris-HCl buffer (50 mM, pH 7.4) for biochemical analysis, while the other was plunged in 10% buffered formol for histological analysis. The number of animals' death was recorded and the percentage of protection (P) was calculated by the relationship:

$$P = (Ns/Nu) \times 100;$$

where Ns is number of survival and Nu a number of mice used.

2.6. Biochemical Analysis

2.6.1. Total Protein Determination

Determination of total protein was performed according to the method of Biuret [18]. Liver homogenate (20%, 10 μL) was added to 1.8 mL of normal saline (NaCl, 0.9%) and 1.5 mL of biuret reagent. The mixture was homogenized, paused for 20 min at 25°C and absorbance was read at 540 nm. The amount of protein in each sample was determined from the bovine serum albumin calibration curve.

2.6.2. Measurement of Liver Enzymes and Bilirubin

The activity of alanine aminotransferase (ALAT) and aspartate aminotransferase

(ASAT) in the sera was evaluated following the protocol of the commercial kit “Fortress Diagnostics Limited”, UK. Alkaline phosphatase (ALP) activity in the mice’s serum was carried out using commercial kit Chronolab Sys S.L., Spain. Total bilirubin in the serum was measured using commercial kit “Fortress Diagnostics Limited”, UK.

2.6.3. Measurement of Nitric Oxide (NO)/Nitrite

The dosage of NO was done in the liver homogenate of D-GaIN/LPS mice, indirectly through the determination of nitrite by Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylene diamine in 2.5% phosphoric acid) [19]. The absorbance of the preparation was measured at 570 nm with the spectrophotometer (Genesis 20) and nitrite level was determined by using the sodium nitrite standard curve.

2.6.4. Malondialdehyde (MDA) Determination

MDA as an indicator of lipid peroxidation in tissues, was determined in the liver of mice by the method described by Wilbur *et al.* [20]. Tissue homogenate (1 ml) were added to 0.5 ml of trichloroacetic acid (20%) and 1 ml of thiobarbituric acid (0.67%). The mixture was allowed to react for 10 min at high temperature (90°C, water bath). The mixture was centrifuged and the absorbance of the supernatant measured at 530 nm. The concentration of MDA was quantified with the extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\cdot\text{cm}^{-1}$ and expressed as μM of MDA per g of protein.

2.6.5. Reduced Glutathione Level

The assay of reduced glutathione was performed following the protocol described by Ellman [21]. Liver tissue homogenates (10 μL) were added to Ellman’s reagent (1500 μL). The mixture was then incubated for 1 h at room temperature and the absorbance was determined at 412 nm. The amount of reduced glutathione (mol/mg of protein) was calculated with molar extinction coefficient (13,600/M \times cm).

2.6.6. Catalase Activity

Catalase activity was evaluated according to the protocol of Sinha [22]. Briefly, 25 μL of homogenate and 375 μL of phosphate buffer (0.1 M, pH 7.5) were mixed. Hydrogen peroxide solution (100 μL , 50 mM) was introduced in the mixture and the reaction was stopped one minute later by adding 1 mL of dichromate/pure glacial acetic acid. All tubes were heated (100°C) for 10 minutes. After cooling, the absorbance was read at 620 nm, catalase activity was determined using the calibration curve and expressed as mmol of H_2O_2 /min/mg of protein.

2.6.7. Superoxyde Dismutase (SOD) Activity

The activity of superoxyde dismutase was evaluated according to the protocol established by Misra and Fridovich [23]. Liver homogenate (67 μL) and carbonate buffer (833 μL , 50 mM, pH 10.2) were added to adrenaline solution (100 μL ,

0.3 mM). The absorbance of the preparation was read at 480 nm, 20 s and 80 s after introduction of adrenaline in the medium. The specific activity of SOD was expressed as SOD unit/mg of protein.

2.7. Histopathological Analysis

The liver samples previously kept in buffered formol (10%) were subjected to the following histological techniques. After dehydration, liver samples were embedded in paraffin (melting point: $56^{\circ}\text{C} \pm 2^{\circ}\text{C}$) to form blocks, 5 μm thick sections from each sample were stained with haematoxylin-eosin and observed under light microscope (Olympus, GHBS, Japan).

2.8. Statistical Analysis

Values are presented as mean \pm standard error by mean (SEM). Statistical differences between control and treated groups were calculated by analysis of variance (ANOVA). Dunnett's post hoc test was also applied using GraphPad In-Stat Software. Values were considered significantly different if $p < 0.05$.

3. Results

3.1. Effect of *M. tomentosa* Methanol Extract on Mortality Rate of Mice Treated with D-GaIN/LPS

One hour after injection of D-galactosamine/lipopolysaccharide (D-GaIN/LPS), the mortality rate was 16.67% in D-GaIN/LPS + distilled water treated mice, two hours after it was 33.33% before it stabilized 4 h later up to the end of the study at 50.00%. In group of mice treated with *M. tomentosa* methanol extract (100 or 200 mg/kg), 3 h after injection of D-GaIN/LPS, the mortality rate was 0.00% and 16.67%, five hours afterward it was 16.67% and 33.33%, respectively, and remained unchanged until the end of experiment. Therefore, the protective effect of *M. tomentosa* methanol extract (100 or 200 mg/kg) was 83.33% and 66.67%, respectively. No death was recorded in animals pre-treated with ascorbic acid (10 mg/kg), which scored a 100% protection.

3.2. Evaluation of Hepatic Function

3.2.1. Effect of *M. tomentosa* Methanol Extract on Liver Enzymes (ALAT, ASAT and ALP) Activity

An increasing activity of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP), of 48.13% ($p < 0.01$), 42.87% ($p < 0.05$) and 162.75% ($p < 0.01$), respectively, compared to normal group, was registered 6h after administration of D-GaIN/LPS to mice. Treatment of animals with *M. tomentosa* methanol extract (100 or 200 mg/kg) or ascorbic acid (10 mg/kg) resulted in a decrease in ALAT, ASAT and ALP activity. The methanol extract (100 mg/kg) induced a significant inhibition of ALAT activity by 23.00%. The activity of ALP was significantly decreased following administration of methanol extract of *M. tomentosa*. The plant extract exhibited 51.55% and 62.20% inhibition of ALP activity at 100 mg/kg and 200

mg/kg, respectively. Ascorbic acid (10 mg/kg) significantly reduced (60.87%) ALP activity. Treatment with the methanol extract (100 or 200 mg/kg) or ascorbic acid kept ASAT activity close to that of normal mice (Figure 1).

3.2.2. Effect of *M. tomentosa* Methanol Extract on Total Protein and Bilirubin Levels

Administration of LPS to D-GaIN sensitised mice decreased total protein liver level by 66.56% ($p < 0.01$), compared to normal control. Plant extract increased (137.88% and 101.02%) protein level ($p < 0.05$) at 100 and 200 mg/kg, respectively, compared to D-GaIN/LPS-treated mice (Figure 2(A)). Pre-treatment of mice with ascorbic acid (10 mg/kg) did not cause a significant increment in total protein content, compared to D-GaIN/LPS mice.

D-GaIN/LPS induced a significant ($p < 0.01$) increase (94.81%) of total bilirubin, compared to normal animals. *M. tomentosa* extract (100 or 200 mg/kg) as well as ascorbic acid (10 mg/kg) exhibited 36.02%, 37.75% and 43.32% inhibition of total bilirubin level, respectively, compared to D-GaIN/LPS-treated mice (Figure 2(B)).

3.3. Evaluation of Oxidative Stress Parameters

3.3.1. Effect of *M. tomentosa* Methanol Extract on Nitrite, MDA and Glutathione Levels

Nitrite level significantly increased from 0.180 ± 0.085 mM in normal mice to 0.886 ± 0.105 mM, 6 hours after D-GaIN/LPS injection. Pre-treatment with ascorbic acid (10 mg/kg) induced a non-significant reduction of nitrite level, compared to D-GaIN/LPS mice. Pre-treatment of mice with *M. tomentosa* methanol extract (100 or 200 mg/kg) did not change the liver nitrite content, compared to D-GaIN/LPS group (Figure 3(A)).

Malondialdehyde level (MDA) increased in significant manner from $18.23 \pm$

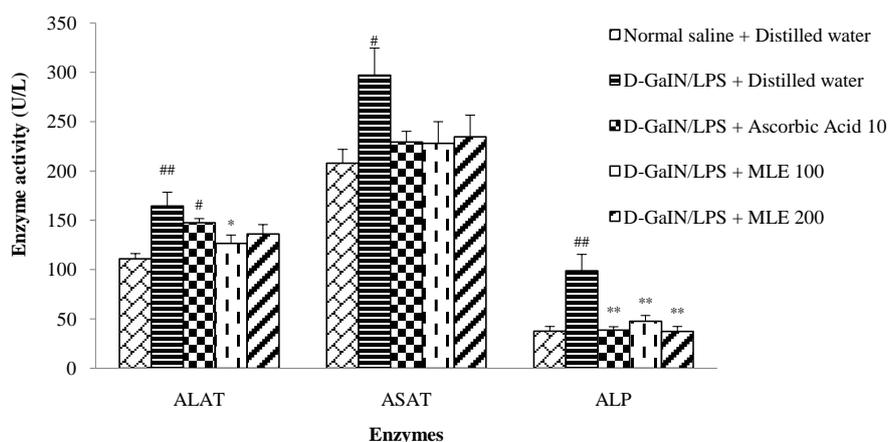


Figure 1. Effect of *M. tomentosa* methanol extract on ALAT, ASAT and ALP serum activity in D-GaIN/LPS-treated in mice. Results are expressed as mean \pm SEM, $n = 6$. * $p < 0.05$; ** $P < 0.01$ significantly different from the D-Gain/LPS group. # $p < 0.05$, ## $p < 0.01$ significantly different from normal group. MLE (100 or 200) = methanol leaf extract of *M. tomentosa* (100 or 200 mg/kg). Ascorbic acid 10 = ascorbic acid (10 mg/kg).

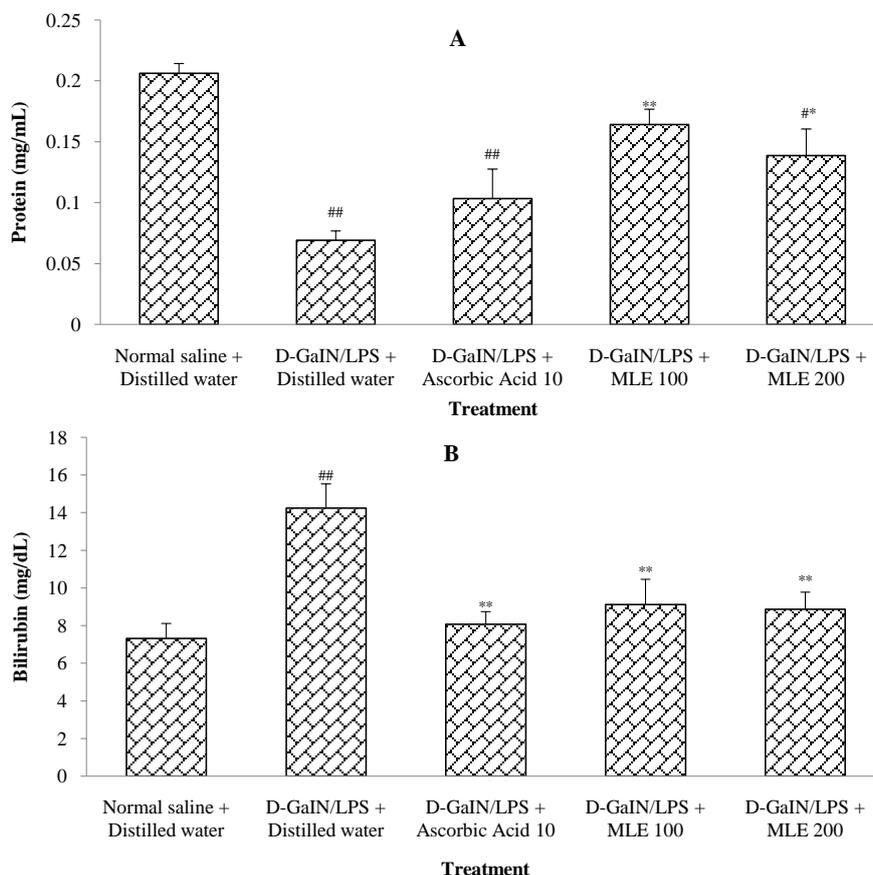


Figure 2. Effect of *M. tomentosa* methanol extract on total protein (A) and bilirubin (B) serum levels in D-GaIN/LPS-induced hepatitis in mice. Results are presented as mean \pm SEM, n = 6. *p < 0.05; **P < 0.01 significantly different compared to D-Gain/LPS group. #p < 0.05, ##p < 0.01 significantly different compared to normal group. MLE (100 or 200) = methanol leaf extract of *M. tomentosa* (100 or 200 mg/kg). Ascorbic acid 10 = ascorbic acid (10 mg/kg).

2.18 nmol/mg of protein in the liver of normal control mice to 69.14 ± 5.95 nmol/mg in the liver of D-GaIN/LPS group. *M. tomentosa* methanol extract significantly inhibited MDA production, exhibiting 62.79% and 48.52% protection at 100 and 200 mg/kg, respectively, compared to D-GaIN/LPS animals. Ascorbic acid (10 mg/kg) administration significantly decreased MDA level by 61.14% compared to D-GaIN/LPS treated mice (**Figure 3(B)**).

Administration of *M. tomentosa* extract or ascorbic acid did not change reduced glutathione level compared to D-GaIN/LPS treated group and normal mice (**Figure 3(C)**).

3.3.2. Effect of *M. tomentosa* Methanol Extract on Catalase and SOD Activities

A significant reduction of catalase activity (67.84%) was recorded 6 h following D-GaIN/LPS administration, compared to normal mice. Administration of ascorbic acid (10 mg/kg) triggered an increase in catalase activity compared to D-GaIN/LPS mice. Administration of *M. tomentosa* methanol extract (100 or

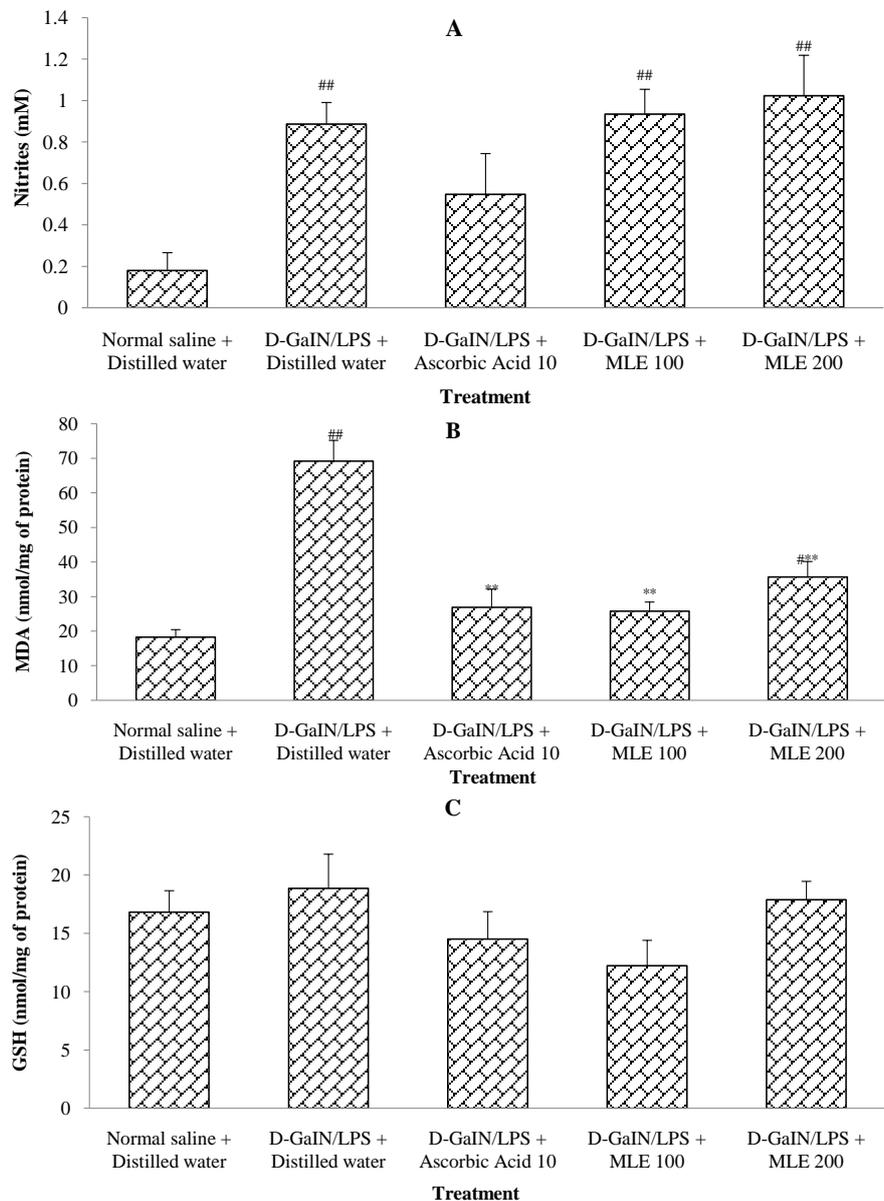


Figure 3. Effect of *M. tomentosa* methanol extract on nitrite (A), MDA (B) and reduced glutathione (C) levels of D-GaIN/LPS-treated mice. Results are expressed as mean \pm SEM, n = 6. **P < 0.01 significantly different compared to D-Gain/LPS group. *p < 0.05, ##p < 0.01 significantly different compared to normal group. MLE (100 or 200) = methanol leaf extract of *M. tomentosa* (100 or 200 mg/kg). Ascorbic acid 10 = ascorbic acid (10 mg/kg).

200 mg/kg) to animals did not induced any significant change in catalase activity in liver tissue, compared to D-Gain/LPS group (Figure 4(A)).

Injection of D-GaIN/LPS resulted 6 h later in a significant ($p < 0.01$) decrease (94.66%) of liver superoxide dismutase (SOD) activity, compared to normal mice. *M. tomentosa* methanol extract (200 mg/kg) and ascorbic acid (10 mg/kg) significantly ($p < 0.01$) increase SOD activity, from 16.17 ± 2.40 units/mg of protein in D-Gain/LPS mice to 127.46 ± 23.56 unit/mg protein and 250.60 ± 42.38 unit/mg of protein, respectively. However, pre-treatment of D-GaIN/LPS

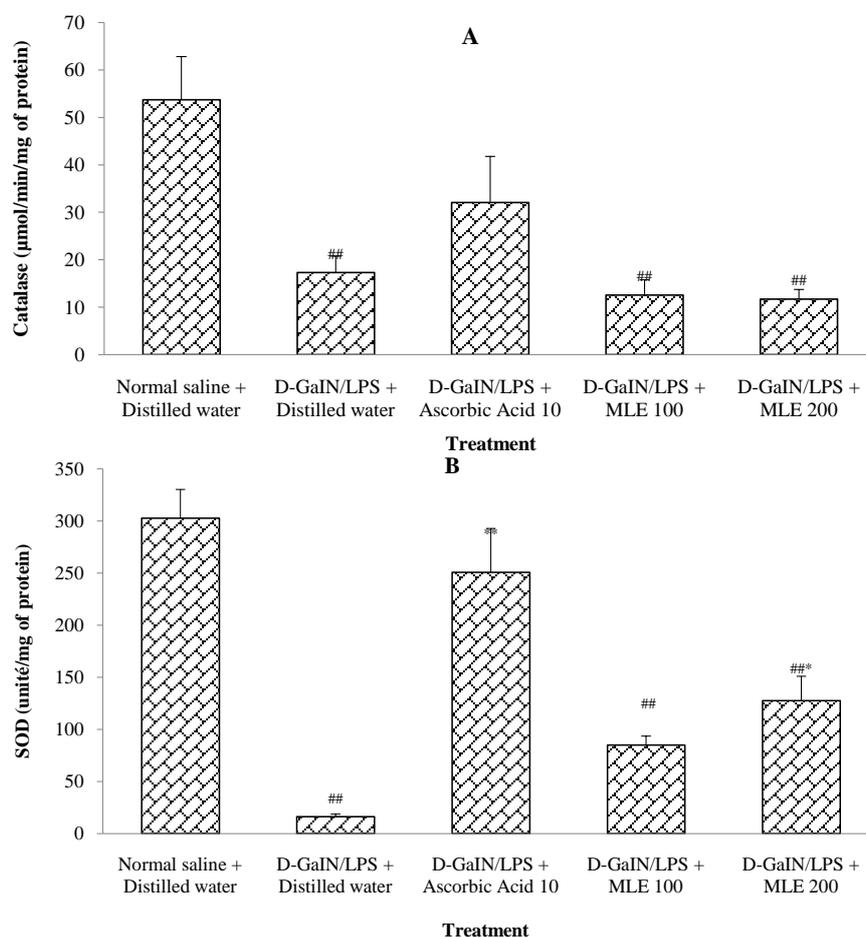


Figure 4. Effect of *M. tomentosa* methanol extract on catalase (A) and SOD (B) activities in the liver of D-GaIN/LPS treated mice. Results are expressed as mean \pm SEM, $n = 6$. ** $P < 0.01$ significantly different from the D-Gain/LPS group. # $p < 0.05$, ## $p < 0.01$ significantly different compared to normal group. MLE (100 or 200) = methanol leaf extract of *M. tomentosa* (100 or 200 mg/kg). Ascorbic acid 10 = ascorbic acid (10 mg/kg).

animals with plant extract failed to completely restore SOD activity to normal level (Figure 4(B)).

3.3.3. Effect of *M. tomentosa* Methanol Extract on D-GaIN/LPS-Induced Liver Damage

Histological examination of the liver of healthy mice showed a steady liver architecture highlighting the veins, arteries and normal liver parenchyma (Figure 5(A)). The histological structure of D-GaIN/LPS mice liver showed a liver parenchyma with many abnormalities such as haemorrhage, hepatocyte steatosis, vascular congestion and many leukocyte infiltrations surrounding blood vessels (Figure 5(B)). Treatment of mice by plant extract (100 or 200 mg/kg) resulted in an improvement of mice liver structure with low leukocyte infiltration, a reduction of steatosis and haemorrhage, compared to D-GaIN/LPS animal (Figure 5(C) and Figure 5(D)). Liver tissue of mice treated with ascorbic acid (10 mg/kg) was less damaged compared to D-GaIN/LPS mice, but was comparable to that of normal mice (Figure 5(E)).

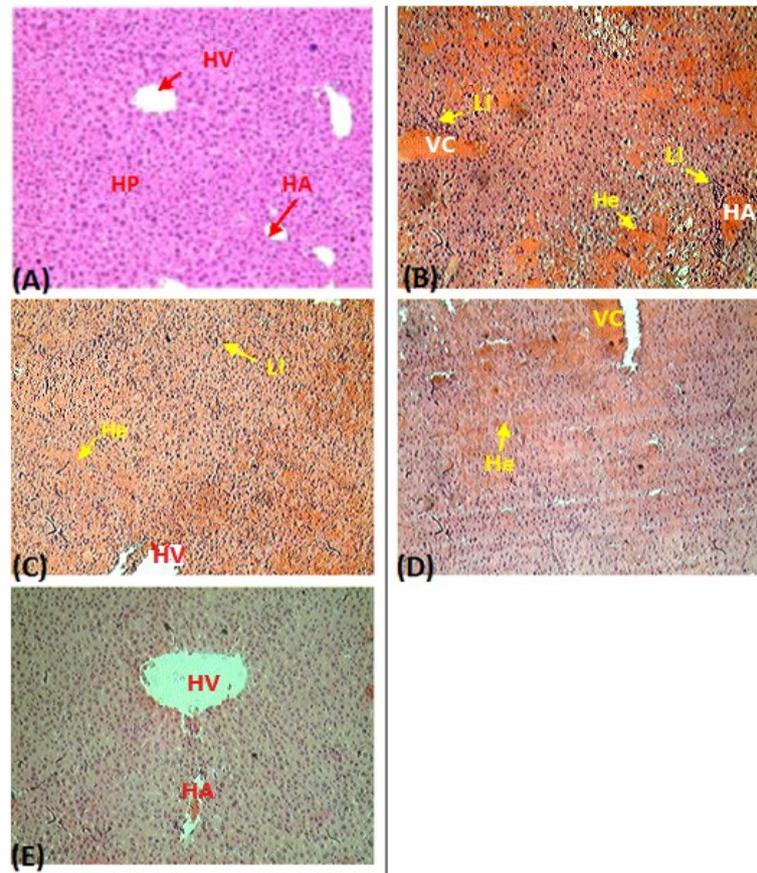


Figure 5. Effect of *M. tomentosa* methanol extract on D-GaIN/LPS-induced liver damage. Haematoxylin-eosin ($\times 100$). (A) Distilled water; (B) D-GaIN/LPS + Distilled water; (C) D-GaIN/LPS + MLE (100 mg/kg); (D) D-GaIN/LPS + MLE (200 mg/kg); (E) D-GaIN/LPS + Ascorbic acid (10 mg/kg); VC: vascular congestion; HA: hepatic artery; HP: hepatic parenchyma; HV: hepatic vein. LI: leukocyte infiltration. He: haemorrhage.

4. Discussion

We investigated the hepatoprotective effects of *Markhamia tomentosa* methanol leaves extract on D-galactosamine/lipopolysaccharide-induced hepatitis in mice. Results showed that, the methanol extract of *M. tomentosa* reduced liver damages caused by D-GaIN/LPS.

Hepatitis induced by injection of D-galactosamine (D-GaIN) and lipopolysaccharide (LPS) is a classical model of acute hepatitis well studied. D-GaIN is a sugar selectively metabolized by hepatocytes. This amino sugar causes a depletion of uridine triphosphate pool and therefore inhibits hepatic synthesis of macromolecules like RNA, proteins and glycogen [24]. This amino sugar induces liver damage and also increases the production of free radical species in hepatocytes [25]. D-GaIN causes liver damage that closely resembles the human viral hepatitis. The hepatitis induced by galactosamine is characterized by increased levels of bilirubin [26]. When D-GaIN is used together with LPS they enhance the development of lethal liver injury or even death of animals. Liver macrophages, when activated by LPS, release a variety of inflammatory cytokines.

D-GaIN/LPS induces excessive production of pro-inflammatory mediators like TNF- α which is released primarily by Kupffer cells and intervenes in the process of apoptosis and necrosis of hepatocytes [27]. Stimulation of inducible nitric oxide synthase by D-GaIN/LPS lead to massive production of NO which contributes to pathogenesis of septic shock and inflammatory diseases such as hepatitis [28]. Reactive oxygen species and other free radicals generally play an important role in the development of many diseases including hepatitis [29].

In this study, administration of D-GaIN/LPS resulted in a significant increase in bilirubin levels compared to the normal. Hepatocytes synthesize bilirubin by catabolism of haemoglobin and excrete it by conjugation reactions with glucuronic acid. This reaction eases elimination of bilirubin in bile or urine. Thus, any increase of bilirubin in the body would indicate a damage of liver function [30]. Treatment of mice with *M. tomentosa* leaves extract and ascorbic acid significantly reduced hepatic bilirubin levels compared to D-GaIN/LPS mice. These results suggest that these substances have preventive effects against D-GaIN/LPS-induced toxicity.

Hepatitis induced by LPS/D-GaIN is characterized by an increased activity of ALAT and ASAT [31]. An increase activity of transaminase is a sign of hepatocytes deterioration [32]. Our results showed a significant increase in transaminase activity after induction of hepatitis, in comparison to normal mice. A significant reduction of hepatic protein levels was observed in D-GaIN/LPS treated mice. A decrease of total protein content of the liver is generally associated with alteration of liver anabolic function [33]. Mice treated by methanol leaves extract (100 mg/kg) displayed a significant reduction of ALAT activity and kept ASAT activity close to the normal. A significant increase in hepatic protein levels was also observed after administration of the plant extract to mice. These results suggest that MLE contain compounds that protect hepatocytes against toxic effects of D-GaIN/LPS. In this study, D-GaIN/LPS caused increase activity of ALP, compared to normal animals. Administration MLE to mice significantly reduced ALP activity compared to D-GaIN/LPS mice, suggesting that MLE preserves from the harmful effects of GaIN/LPS in mice liver.

Injection of D-GaIN/LPS induces overproduction of inflammatory substances like TNF- α , IL-1 and NO. These mediators are involved in apoptosis or necrosis of hepatocytes [27] [34]. Overproduction of NO can activate nuclear factor kappa B, which will induce the expression of pro-inflammatory mediators (TNF- α , IL-1) and cause oedema by increasing vascular permeability [35] [36]. It also contributes to peroxynitrite production, causing nitration and oxidation of proteins, blockage of mitochondrial function, DNA damage, cell apoptosis or necrosis associated to inflammatory diseases [37] [38]. Nitrite level was significantly increased in hepatic tissue of D-GaIN/LPS mice compared to normal animals. Pre-treatment of mice with MLE did not significantly change nitrite level in D-GaIN/LPS-treated mice. We have previously demonstrated that MLE significantly decreased, in dose-dependent manner, serum, spleen and liver nitrite content in formalin-induced chronic inflammation [15]. These results

may be due to subacute treatment with MLE. No structural alteration was observed in the liver parenchyma of normal mice. However, D-GaIN/LPS induced haemorrhage, steatosis, necrosis and leukocyte infiltration in liver tissue. Pre-treatment with MLE and ascorbic acid reduced damage caused by D-GaIN/LPS in liver tissue, which was similar to that of normal mice. These observations suggest the protecting action of MLE against D-GaIN/LPS induced liver damage.

It has been reported that, free radicals species interact with polyunsaturated fatty acids and increase lipid peroxidation, leading to production of malondialdehyde (MDA), which is commonly used as an indicator of liver damage [31]. Results showed an elevated rate of MDA in liver tissue of mice treated with D-GaIN/LPS. Treatment with MLE significantly decreased MDA levels, suggesting an antioxidant activity of this extract.

Superoxide dismutase (SOD), catalase and glutathione peroxidase are the main antioxidant enzymes that protect the body against oxidant stress [39]. SOD limits accumulation of superoxide anion ($O_2^{\cdot-}$) and generates hydrogen peroxide (H_2O_2) involved in production of hydroxyl radical (HO^{\cdot}) by Fenton reaction. Catalase catalyses the conversion of H_2O_2 into oxygen (O_2) and water (H_2O). Glutathione peroxidase uses reduced glutathione as a substrate to degrade organic hydroperoxydes and H_2O_2 into harmless compounds. These enzymes control the balance between pro-oxidant and antioxidant processes. MLE (200 mg/kg) and ascorbic acid instigated a significant raise in SOD activity. These results show that MLE would have an inhibitory effect on pro-oxidant process.

5. Conclusion

The aim of the present work was to assess the effect of *Markhamia tomentosa* methanol leaves extract on D-galactosamine/lipopolysaccharide-induced acute liver injury in mice. Administration of plant extract before injection of D-GaIN/LPS reduced ALAT and ALP serum activity, bilirubin and MDA liver levels and increased SOD activity and total protein level, compared to animal treated with D-GaIN/LPS and vehicle. Histological analysis also revealed that MLE prevented D-GaIN/LPS to induced liver injury in mice. The present study demonstrates that *Markhamia tomentosa* methanol extract may have protective effects against hepatitis. MLE protects against D-GaIN/LPS-induced fulminant hepatic failure through inhibition of tissue peroxidation, reduction of oxidant stress and thus, prevents tissue damage. MLE could offer an alternative treatment for acute liver inflammation. The precise mechanisms of action of MLE and its ability to provide a preventive effect during D-GaIN/LPS-mediated liver failure are still to be investigated.

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Conflicts of Interest

The authors certify that they have no affiliation with, or financial involvement in any organization with a direct financial interest in the subject matter or materials discussed in the manuscript.

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