Original Research Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

HEPATOPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF NEW LECTIN PURIFIED FROM *Morus nigra* AGAINST LIPOPOLYSACCHARIDE-INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT

The present study was undertaken to evaluate the protective effect of lectin purified from *Morus nigra* against lipopolysaccharide induced oxidative stress in experimental rats. Exposure of rats to lipopolysaccharide caused a significant increase in the lipid peroxidation level along with corresponding decrease in the reduced glutathione and various antioxidant enzymes in liver.and increase in serum: APL and transaminases activities. Supplementation of purified lectin of *Morus nigra* to lipopolysaccharide decrease in the serum the activities of AST, ALT and APL along with increase in liver GSH level and various antioxidant enzymes: glutathione peroxidase (GSH-Px), glutathione –S-transferase (GST) and catalase however the level of lipid peroxidation was increased in liver. Furthermore, no histological alterations were observed in the liver of lectin of *Morus nigra*. The results clearly demonstrate the protective role of new lectin purified from *Morus nigra* against LPS induced inflammatory liver in rats.

KEYWORDS: ***Antioxidant enzymes, lectin, lipopolysaccharide, anti-inflammatory activity, Morus nigra.***

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Received on: 26-07-2017
Revised and Accepted on: 07-12-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2018.9.1.p66-72

INTRODUCTION

Lipopolysaccharide (LPS), an endotoxin, is a major glycolipid component of the outer cell wall of gram-negative bacteria, made up of a polysaccharide O-chain and a biologically active lipid-A moiety, embedded within the bacterial membrane[1]. Endotoxemia-induced toxicity is characterized by injury to various organs, including liver, kidney and the brain, and it has been implicated as a contributing factor to bacterial infection resulting in sepsis, which is one of the major causes of morbidity and mortality in intensive care units[2]. Most of the toxicities observed in LPS-induced injury in the kidney and systemic circulation has been attributed to toxic mediators produced by activated macrophages, including cytokines, such as tumor necrosis factor-α (TNF-α), interleukins (IL-1, IL-6, IL-8, and IL-12), other proinflammatory molecules, including platelet-activating factor, prostaglandins, as well as reactive oxygen and nitrogen species (RONS), such as nitric oxide (NO) and superoxide radical. The systemic inflammatory response triggered in the host by LPS is characterized by fever, leukocytosis, thrombocytopenia, changed metabolic responses and redox status impairment[2]. The consequences of impaired intracellular redox balance includes the generation of excessive RONS, induction of lipid peroxidation, DNA and protein damage, depletion of intracellular stores of endogenous antioxidants and inhibition of antioxidant enzymes[3]. The involvement of oxidative stress in injury associated with LPS suggests that dietary antioxidants may enhance the efficacy of treatment protocols designed to mitigate LPS-induced endotoxemia. Medicinal plants, fruits, vegetables, spices and teas are drawing a lot of attention because of their demonstrated health benefits, with scientific evidence demonstrating that phytochemicals in fruits, vegetables, spices and teas possess a high number of protective biological properties, including antioxidant, anti-inflammatory and other beneficial effects[4]. Lectin or glycoproteins are carbohydrate binding proteins that interact with specific sugars and induce several biological activities. Lectin has attracted great research due to its various biological activities like cell agglutination, antitumor, immune modulatory, antifungal, antiproliferative and antiviral activities These proteins were powerful antioxidants which could inhibit lipid peroxidation and scavenge free radicals[5].The purpose of this study was to evaluate the protective role of a new lectin purified from the roots of *Morus nigra* collected from Algeria against LPS induced inflammation in rat's liver.

MATERIAL AND METHOD

The lectins isolation from root of *Morus nigra* used in this work originated from Algerian.

Lectin Purification by stroma column

The root of plant of *Morus nigra,* was washed briefly, roughly ground and then homogenized in a chiled warning blender with phosphate buffer saline pH7.2. The homogenized was then centrifuged at 6000 rpm for 30 min; the remaining debris was removed by passing the supernatant through filter paper[6]**.** Erythrocyte membranes (stroma) were obtained by lysis of rabbit red blood cells. The membranes were fixed with 1% glutaraldehyde overnight at 4°C, then the stroma were washed with distilled water and freeze dried. Two hundred milligrams of erythrocyte stroma was physically entrapped in a chromatographic column with Sephadex G-25. The column was equilibrated with SSI (0.15 M NaCl). The crude extract (160 mg of protein) was applied to the stroma column (10 x 1.2 cm), and the unretained material was eluted with SSI at a flow rate of 1 mL, until the A280 of the collected fractions was below 0.01. The bound lectin was eluted with acetic acid (3%) and the pH of each collected fraction was adjusted to 6. All fractions collected were lyophilized and used for determination of anti-inflammatory activity in vivo.

Determination of Anti-inflammatory activity of Morus nigra in vivo

All chemicals used in this work were purchased from Sigma chemical company. Laboratory animals, *Albino wistar* male rats, were brought from the Algiers Pasteur institute at the age of 4 weeks, with an average live weight of 160g. They were located in a room with an ambient temperature of 21±1°C and up to 12h of light daily. The rats were divided into six experimental groups, each consisting of six rats.

Group 1: served as the control, treated with normal saline.
Group 2: treated with reference drug diclofénac at a dose of 3mg/kg for 14 days.
Group 3: treated with purified lectin only at a dose of 15mg/kg byintraperitoneal injection for 14 days.
Group 4: treated with lipopolysaccharide at a dose of 200µg/kg by intraperitoneal injection for 14 days.
Group 5: treated with reference drug diclofénac at a dose of 3mg/kg 30 min before lipopolysaccharide injection for 14 days.
Group 6: treated with purified lectin at a dose of 15mg/kgbyintraperitoneal injection 30 min before lipopolysaccharide injection for 14 days.

Twenty four hours after the last administration the blood was collected by retro- orbital sinus punction from each anesthetized rat. After centrifugation at 3000 rpm for 10min, the serum was separated immediately and stored at– 20°c until determination of: enzymes (AST, ALT and ALP) activities. Subsequently, rats were decapitated and livers were removed.

Tissue preparation

About 500mg of liver was homogenized in 4ml of buffer solution of phosphate buffered saline (w/v: 500mg tissue with 4ml PBS, PH 7.4) homogenates were centrifuged at 10.000xg for 15min at 4°c. The resultant supernatant was used for determination of: reduced GSH, Thiobarbituric acid- reactive substance (TBARS) levels, and the activities of: GSH-PX, GST and catalase[7].

Determination of C-Reactive Protein (CRP) level and enzymes

CRP level and SerumAST, ALT and ALP activities were determined using automated analyses.

Determination of lipid peroxidation (LPO)

Lipid peroxidation level in the liver was measured by the method of Buege and Aust (1978)[8]. 125µl of

supernatant were homogenized by sonication with 50 µl of PBS, 125 µl of 20% TCA + BHT 1% (TCA-BHT) in order to precipitate proteins, and centrifuged (1000xg, 10min, 4°c), afterwards, 200µl of supernatant were mixed with 40µl of HCl (0,6M), and 160µl of TBA dissolved in tris (120 mM). And the mixture was heated at 80°c for 10min; the absorbance was measured at 530nm. The amount of TBARS was calculated by using a molar extinction coefficient of $1.56x10^5$ M/Cm.

Determination of reduced glutathione (GSH)

GSH content in liver was measured spectrophotometrically by using Ellman's reagent (DTNB) as a colouring reagent, following the method described by Weeckbekeretcory (1988)[9].

Determination of glutathione-S-transferase (GST) (EC2.5.1.18)

The cytosolic glutathione-S-transferase activity was determined spectrophotometrically at 37°c by method of Habig et al (1974)[10]. The reaction mixture (1ml) contained 0.334ml of 100mM phosphate buffer (PH 6.5), 0.033ml of 30mM CDNB and 0.33ml of reduced Glutathione. After pre-incubating for 2min, the reaction was started by adding 0.01ml of diluted cytosol and the absorbance was followed for 3min at 340 nm. The specific activity of GST is expressed as µmole of GSH-CDNB conjugate formed/ min /mg protein using extinction coefficient of 9.6 Mm^{-1} cm^{-1}

Determination of GSH-Px (E.C.1.11.1.9)

Glutathione peroxidase (EC 1.11.1.9) activity was modified from the method of Flohe and Gunzler (1984) [11]. for the enzyme reaction, 0.2ml of the supernatant was placed into a tube and mixed with 0.4ml GSH (reduced glutathione, sigma product, analytical grade), and the mixture was put into an ice bath for 30min. Then the mixture was centrifuged for 10min at 3000rpm, 0.48ml of the supernatant was placed into a cuvette, and 2.2ml of 0.32M Na_2HPO_4 and 0.32ml of 1m mol/l 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, sigma) were added for color development. The absorbance at wavelength 412nm was measured with a UV spectrophotometer after 5min. The enzyme activity was calculated as a decrease in GSH within the reaction time as compared with the non-enzyme reaction.

Catalase activity (CAT)

The activity of catalase (CAT) (E.C.1.11.1.6) was measured according to the method of Aebi (1984)[12] The reaction mixture 1ml contained a 100mM phosphate buffer (pH 7), 500mM H_2O_2 and liver supernatants. The reaction started by adding H_2O_2 and its decomposition was monitored by following the decrease in absorbance at 240nm for 1 min. The enzyme activity was calculated by using an extinction coefficient of 0.043mM-1cm-1.

Protein quantification

Protein was measured by the method of Bradford (1976)[13], using bovine serum albumin as the standard.

Histopathological examination

Liver from autopsied animals were excised out and fixed in formalin (10%). Sections of five micron thickness were prepared by using microtome and these sections were stained with hematoxyline and eosin. For histological alterations these slides were observed under light microscope [7].

Statistical analysis

The data was subjected to student *t* test for comparison between groups. The values were expressed as mean ± SEM. Significance level was set at $P<0.05$, $P<0.01$, $P<0.001$.

RESULTS

Effects of treatments on serum biochemical Parameters

A very highly significant ($P\leq0.001$) elevation in serum the activities of AST, ALT and ALP was observed in lipopolysaccharide intoxicated rats. Only purified lectin or diclofenac treatment did not show any significant alteration. However, the combined treatment of lectin or diclofenac with lipopolysaccharide show no significant decline in serum AST, ALT and ALP activities was noticed respect to controls (table 1).

Effects of treatments on hepatic oxidative stress parameters

Lipopolysaccharide exposure rats a highly significant ($p\leq0.01$) depleted in reduced glutathione level, SOD, GSH-Px GST and catalase activities. And no significant increase in liver lipid peroxidation level in lipolysaccharide intoxicated rats was noticed. Diclofenac or purified lectin alone treatment did not show any significant decline. In combined treatment of lipopolysaccharide with diclofenac or purified lectin there was no significant increase in reduced glutathione level, SOD, GSH-Px GST and catalase activities. And no significant depletion in lipid peroxidation level was recorded with respect to the control (Figure 1 and 2).

Histological studies

Lipopolysaccharide induces various pathological alterations in liver of rats. These alterations were characterized by centrilobular necrosis, degranulation, destruction of membrane cells, cytoplasmic vacuolization (Fig. 3C). Combined treatment of lipopolysaccharide with diclofenac or purified lectin showed reparative changes. Liver showed prominent recovery in the form of normal hepatocytes and very less centrilobular necrosis. Pronounced sinusoid with granular hepatocytoplasm were also evident (Fig.3D). Liver of the control group had a regular histological structure with a characteristic pattern of hexagonal lobules (Fig. 3A). Furthermore, no histological alterations were observed in the liver of diclofenac or purified lectin treated group (Figure 3B).

Table 1

Changes in biochemical parameters of control and rats treated with lectin, diclofenac, lipopolysaccharide (LPS) and combined treatment of LPS with lectin or Diclofenac after 14 days of treatment.

	Control	Lectin	Diclofenac	LPS	Lectin+LPS	Diclofenac+LPS
AST(U/L)	132.16±3.9	145.6±4.5	143.2±3.2	217.4±2.	174.4±3.7	167.4±3.9
ALT (U/L)	35.2±0.03	36.3±0.02	34.5±0.03	60.2±0.02***	45.96±0.01	40.93±0.01
PAL (U/L)	302.3±0.22	302.8±0.2	304.6±0.1	385.7±0.4***	343.5±0.3	307.5±0.4
CRP	0.29±0.03	0.3±0.02	0.28±0.03	0.5±0.03***	0.44±0.04	0.42±0.02

Values are given as mean ± SEM for group of 6 animals each. *P≤0.05, compared to controls. **P≤0.01, compared to controls. ***P≤0.001, compared to controls.

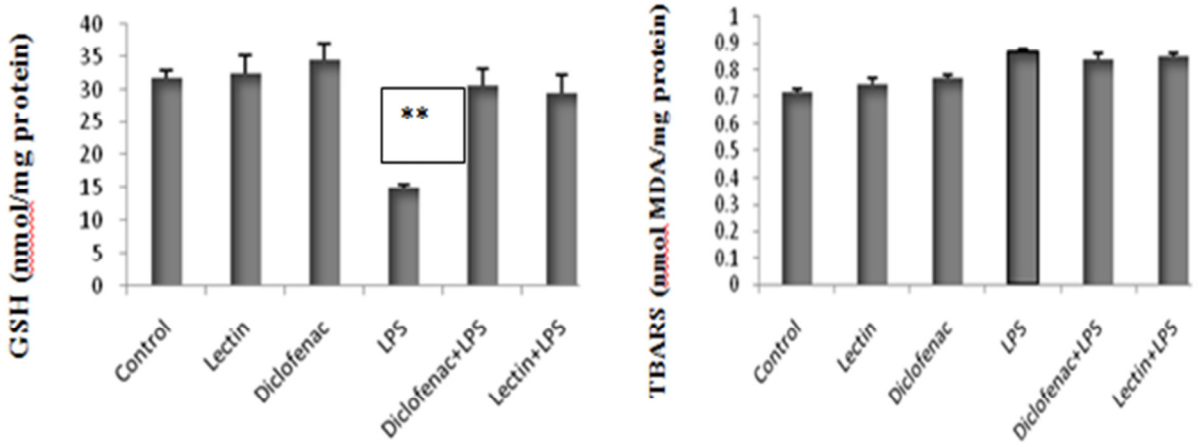
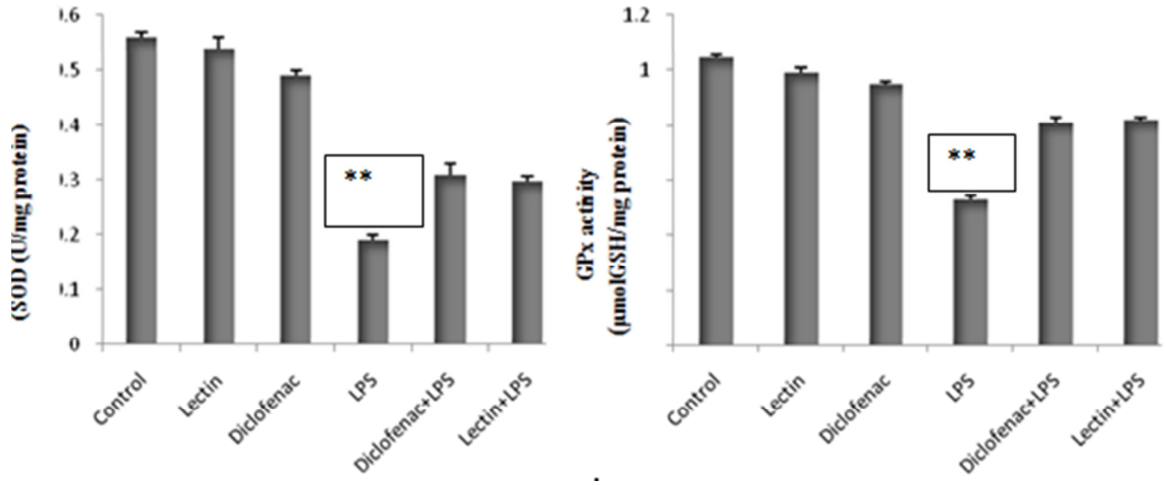


Figure 1

Reduced glutathione (nmol/ mg protein) and TBARS (nmol MDA /mg protein) levels in liver of control and rats treated with Lectin, Diclofenac, LPS and combined treatment of LPS with Diclofenac or Lectin after 14 days of treatment. Values are given as mean ± SEM for group of 6 animals each significant difference: *compared to controls (*P≤0.05; **P≤0.01; ***P≤0.001).



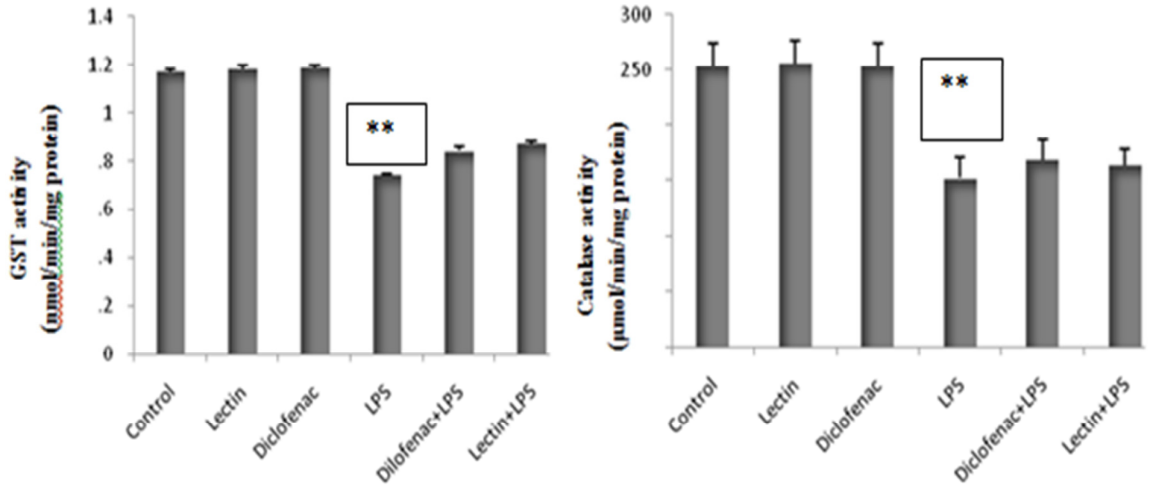


Figure 2

Enzyme activities of SOD (U/mg protein), GPx (µmol GSH/ mg protein), GST (nmol /min/mg protein) and Catalase (µmol/min/mg protein) in liver of control and rats treated with Lectin, Diclofenac, LPS and combined treatment of LPS with Diclofenac or Lectin after 14 days of treatment. Values are given as mean ± SEM for group of 6 animals each significant difference: * compared to controls (*P≤0.05; **P≤0.01; ***P≤0.001).

DISCUSSION

In the present study, oxidative stress induced by LPS was evidenced in kidney of rats by increase in lipid peroxidation level and the inhibition of SOD, GSH-Px, GST and catalase activities. As consequence of lipid peroxidation biological membranes are affected causing cellular damage. In the present study, serum AST, ALT and PAL activities were significantly increased after 14 days LPS (200µg/kg), showing insufficiency of liver function. The alterations in liver function in LPS treated rats may also be secondary to ROS(reactive oxygen species), which induce contraction of mesangial cells The activity of SOD, GSH-Px, GST and Catalase that can clear to protect the cells from being injured represents the competence of clearing free radicals from the organism. MDA content manifests the level of lipid peroxidation. From GSH, MDA levels and SOD, GSH-Px, GST and Catalase activities in liver of rats. LPS alone significantly decreased GSH level, SOD, GSH-Px, GST and Catalase activities and increased MDA content along with histological damage in liver. The focus of this study was to investigate the acute effects of LPS-induced hepatic oxidative stress and inflammatory responses, and the possible protection offered by administration of purified lectin of *Morus nigra*. Oxidative stress is a well-known mechanism of LPS induced hepatic injury, and the redox imbalance produced may result in depletion of endogenous antioxidants such as the antioxidant enzymes and alteration of GSH redox status. Thus, augmenting the antioxidant defense system becomes necessary, especially during infections or periods of chronic oxidative insult. Whole extracts or isolated compounds from plants are popular applications to reverse and/or prevent liver toxicity and oxidative stress produced by noxious agents, such as LPS and these beneficial effects may be attributed to their antioxidant and anti-inflammatory properties. In this study, it was observed that injection of LPS resulted in liver injury as indicated by an elevation in the levels of serum AST, ALT and PAL, all circulating markers of liver injury[14-15]. Results from the current study showed that supplementation with the purified lectin of *Morusnigra* for 30 min prior to the LPS injection, reversed the induced damage in the liver. The protective effect of purified lectin of *Morusnigra* observed in our study may be due to the ability of the proteins to stabilize and maintain the integrity of the liver membrane, as well as repair damaged liver tissues by stimulating liver cells regeneration and protein synthesis. LPS-induced lipid peroxidation is an index of oxidative stress, and several previous studies have reported enhanced lipid peroxidation in many tissues (including liver, heart, brain, small intestine and stomach) of rats.[14,16] Under conditions of oxidative stress, reactive oxygen and nitrogen species (RONS) attack the polyunsaturated fatty acids (PUFAs) of cell membranes causing destabilization, disintegration and alteration in membrane fluidity and permeability, all events which increase the rate of protein degradation and eventually lead to cell lysis[15]. Decomposition products of lipid hydroperoxides such as MDA and 4-HNE can also cause chaotic cross-linkage with protein and nucleic acids, leading to oxidative protein and DNA damage[15]. In this study, hepatic MDA, as markers of lipid peroxidation, was measured. Elevated levels of hepatic MDA were observed. Pre-feeding lectin of *Morus nigra* for 14 days in the LPS-challenged rats inhibited the formation of MDA in the liver. A large number of in vitro studies have established the excellent free radical scavenging ability of lectin purified from *Morus nigra* and this has been confirmed in many in vivo studies.. The modulation of the activities of the antioxidant enzymes observed in the LPS-injection rats consuming lectin of *Morus nigra* could be ascribed to the direct quenching of RONS generated by LPS, since antioxidant activity of lectin is established to be free of radical scavengers. Furthermore, the up-regulation and/or down regulation of the gene expression of the antioxidant enzymes may be an additional mechanism that should be elucidated in future studies. Reduced glutathione (GSH) is the major non-protein thiol in plant and animal cells. It is essential for the regulation of a variety of cellular functions,

playing an important role in intracellular protection against ROS and other free radicals [17]. Because of its sulphydryl (–SH) group, it can function as a nucleophile, forming conjugates with many xenobiotics and/or their metabolites and also serve as a reductant in the metabolism of hydrogen peroxide and other organic peroxides [18] . During interaction with free radicals, the –SH group of GSH becomes oxidized, leading to the formation of corresponding disulfide compound (GSSG). Thus, a depletion of GSH is usually associated with an increase in GSSG concentration and a lowered GSH: GSSG redox ratio during conditions of oxidative stress [19, 20]. Results from the current study revealed a decrease in GSH in rats injection with LPS. These events invariably resulted in a decrease in the GSH in both tissues of LPS-challenged rats. Feeding lectin to LPS-treated rats, restored the GSH to values comparable to those found in the negative control animals, indicating that lectin purified for *Morusnigra* is able to protect against LPS-induced glutathione imbalance. This result is supported with biochemical and histopathological findings which the effect of lectin on lipopolysaccharide induced oxidative stress in rats.

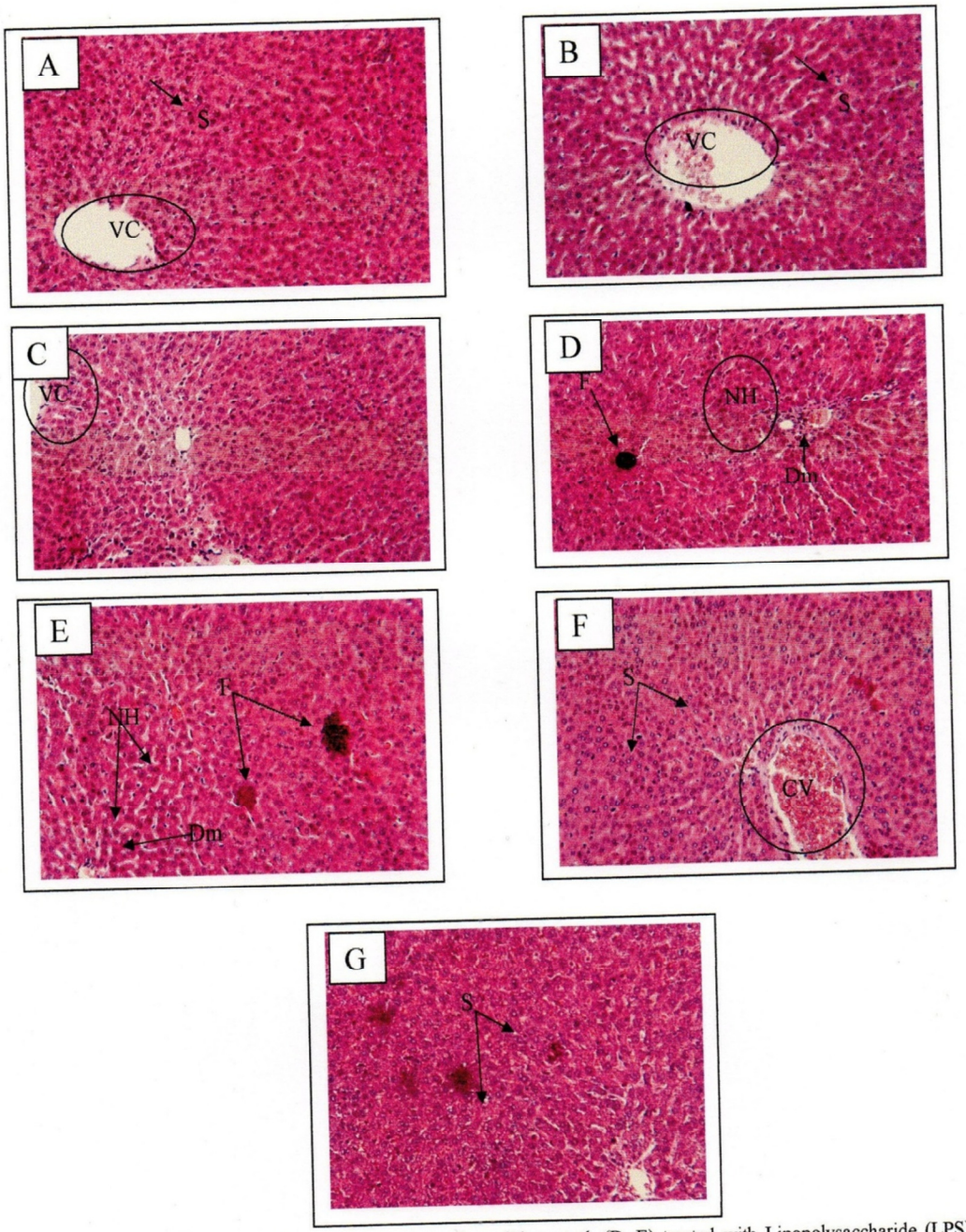


Fig. 3. Microscop evaluation of hepatic tissue from (A) control, (D, E) treated with Lipopolysaccharide (LPS) alone, and in combination with Diclofenac or Lectin (B,C) . Section were stained using the hematoxylin-eosin method (100X). Bn: Bright nuclei, Ds: destruction of membrane cells, N: Necrosis, INTH: Intact hypatocyte cells, CV: central vein, S: Sinusoid. (F, G) Diclofenac or Lectin coadministrated with Lipopolysaccharide shows granular cytoplasm and normal hepatocytes.

CONCLUSION

This study provides the first in vivo evidence of an anti-inflammatory effect of lectin purified from *Morusnigra* in LPS-induced renal injury in rats. LPS-induced the production of reactive oxygen and nitrogen species, resulting in lipid peroxidation as demonstrated in this study. Results from this study further demonstrates that lectin purified from Morusnigra is able to suppress LPS triggered oxidative stress and inflammatory responses in the liver by attenuating liver damage, lipid peroxidation, and redox (GSH:GSSG) imbalance in a Wistar rat model. Lectin purified from Morusnigra has excellent antioxidant properties which may in part, explain this observed anti-inflammatory activity. This suggests that Lectin purified from Morusnigra may be of benefit in the prophylactic management of LPS-induced liver injury, however, future studies are necessary to fully examine the specific mechanisms underlying the protective effects shown by this purified Lectin.

CONFLICT OF INTEREST

Conflict of interest declared none.

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