Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: http://www.tandfonline.com/loi/iphb20

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To cite this article: R. Rajamurugan, A. Suyavaran, N. Selvaganabathy, C. H. Ramamurthy, G. Pramodh Reddy, V. Sujatha & C. Thirunavukkarasu (2012) Brassica nigra plays a remedy role in hepatic and renal damage, Pharmaceutical Biology, 50:12, 1488-1497, DOI: 10.3109/13880209.2012.685129

To link to this article: https://doi.org/10.3109/13880209.2012.685129

Published online: 14 Sep 2012.

Pharmaceutical Biology, 2012; 50(12): 1488–1497

ISSN 1388-0209 print/ISSN 1744-5116 online
DOI: 10.3109/13880209.2012.685129

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RESEARCH ARTICLE

Brassica nigra plays a remedy role in hepatic and renal damage

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Abstract

Context: Black mustard [*Brassica nigra* (L.) Koch] of the Brassicaceae (Cruciferae) family is commonly used as a spice and a cheap source of antimicrobial agents for bacterial infections.

Objectives: The present investigation was to demonstrate the protective effect of the methanol extract of *B. nigra* leaves against D-galactosamine (D-GalN)-induced hepatic and nephrotoxicity in Wistar rats.

Methods: Activity of the methanol extract of *B. nigra* at doses of 200 and 400 mg/kg b.wt. against D-GalN (500 mg/kg b.wt.) induced toxicity, with silymarin used as the standard. Histological damage, activities of serum marker enzyme, hematological changes, metabolites such as bilirubin, urea, uric acid, and creatinine levels, tissue thiobarbutric acid reactive substance, enzymic and non-enzymic antioxidants and inflammatory marker enzymes such as myeloperoxidase, cathepsin D, and acid phosphatase were assessed.

Results: The D-GalN-induced toxicity was evident from a significant increase ($p < 0.001$) in the serum and tissue inflammatory markers in toxic rats, when compared with the control (saline alone treated animals). The *B. nigra* pretreated groups (200 and 400 mg/kg b.wt.) showed significant ($p < 0.001$) reduction in the D-GalN-induced toxicity as obvious from biochemical parameters. Histopathological observations confirm the protective effect of *B. nigra* leaf extract by reduction in hepatic and renal tissue damage. Experimentals extract showed a similar effect as the standard.

Conclusions: The crude methanol extract of *B. nigra* leaf lacks inherent toxicity and exhibits hepatic and nephroprotective effects against D-GalN-induced toxicity in Wistar rats.

Keywords: Antioxidant, D-galactosamine, lipid peroxidation, oxidative stress, phytochemistry

Introduction

The liver is an indispensible exocrine organ of the human body which performs detoxification of various xenobiotics such as drug metabolites and alcohol and helps in maintaining homeostasis (Rathi et al., 2008). During the detoxification process, liver cells frequently experience stress due to oxidative damage from free radicals. Progressive impairment of liver cells due to oxidative stress would lead to life threatening complications if left untreated (Wolf, 1999). Anand et al. (2002) reported D-galactosamine-induced acute liver failure results in renal damage and is mediated by endothelin receptors. Recently, the term hepatorenal syndrome (HRS) has been introduced to define the development of renal failure related with hepatocellular dysfunction (Epstein, 1996). Although several chemosynthetic products are recommended for liver therapy and to prevent renal damage most of them prove

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(Received 19 December 2011; revised 11 April 2012; accepted 11 April 2012)

to be immunosuppressive. To solve this problem, studies on novel therapeutic strategies using plant product based drugs are in the process of vigorous testing (Liu & Xiao, 1994; Lin et al., 1998; Venkateswaran et al., 1998; Gerbes et al., 2006).

Brassica nigra (L.) Koch (Brassicaceae) is commonly known as black or brown mustard (Nair & Henry, 1983). The black mustard has a stronger pungent flavor than the white and brown species. *B. nigra* has been used in traditional herbal medicine for a long time, especially the seeds as rubiefacient poultice. *B. nigra* is cultivated worldwide. In addition to its significance as a food flavoring ingredient, the seeds of *B. nigra* have important medicinal uses in treatment of rheumatism and joint pains, indurations of the liver and spleen, throat tumors and as a laxative (Gerald & Williams, 1989; Obi et al., 2009). Recent reports revealed that *B. nigra* was found to have antioxidant and antimicrobial activities (Obi et al., 2009; Hussein et al., 2010). Although the therapeutic properties of *B. nigra* seeds have been clearly elucidated in earlier studies, there is little scientific information about the pharmacological activities of leaves of the plant. Recently, we studied *in vitro* antioxidant property and phytochemical constituents of the *B. nigra* leaf extract (Rajamurugan et al., 2011). The HPTLC analysis of *B. nigra* leaf extract quantitatively revealed the presence of phenolics compounds such as gallic acid (4.31 mg/g), quercetin (0.91 mg/g), ferulic acid (0.76 mg/g), caffeic acid (0.55 mg/g) and rutin (0.36 mg/g). The GC-MS analysis showed that *B. nigra* extract consists of a mixture of bioactive compounds such as α-amyritin, L-proline, 5-oxo-methyl ester, 1,2-benzenedicarboxylic acid, diisooctyl ester, 1,4-dichloro-benzene, etc. (Rajamurugan et al., 2011). Hence, the present study evaluated hepatic and nephroprotective effects of the leaf extract against D-GalN-induced liver and nephrotoxicity in Wistar albino rats.

Materials and methods

Experimental animals

Adult albino Wistar rats of weight variation not exceeding ±20% of the mean weight (130–160 g) were selected for the present study. Animals were procured from Tamil nadu Veterinary and Animal Sciences University, Madhavaram milk colony, Chennai, India. All the animals were acclimatized to laboratory conditions for a week before the commencement of experiments. The animals were housed in polycarbonate cages (three rats in each cage) at controlled room temperature of 28–30°C and a relative humidity between 30 and 70% and a constant 12 h light:dark cycle. The rats had free access to water and dry rat pellets *ad libitum*. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and the experimental protocol was approved by the institutional animal ethics committee (CPCSEA/CRIS/PHARMA/54/2008).

Plant material and extraction

Plant material (*B. nigra* leaves) was collected between October and November 2008, around the villages of Panruti, Postal code-607805, Tamil Nadu, India and authenticated by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, National Institute of Herbal Science, Chennai, South India (PARC/2008/560).

About 500 g of the shade-dried, powdered leaves of *B. nigra* was exhaustively extracted in a Soxhlet extractor by continuous hot percolation with methanol. The residue was filtered and concentrated under reduced pressure and the extract (yield = 11.2%) was used for evaluating hepatic and nephroprotective effect.

Chemicals

Phenazonium methosulphate, nitroblue tetrazolium (chloride), nicotine adenine dinucleotide hydride, thio barbituric acid, butylated hydroxyltoluene, sodium azide, D-GalN, reduced glutathione (GSH), were purchased from Sigma Chemical Co., St. Louis, USA. Tris-HCl buffer, 5,5′-dithiobis 2-nitrobenzoic acid, 2,4-dinitrophenylhydrazine, ascorbic acid, sodium acetate buffer, hexadecyl trimethyl ammonium bromide, *O*-dianizidine and all other chemicals and solvents were of analytical grade and obtained from S.D. Fine Chemicals, Mumbai, India. Kits were obtained from Accurex Biomed Pvt Ltd and BAYER Pvt Ltd, India.

Hepatoprotective activity study

Animals were divided in to six groups of six animals each (comprising three males and three females). Group I animals served as control and received only vehicle (saline) for 21 days. Group II animals served as toxic control received vehicle (saline) for 21 days. Groups III, IV and V were prophylactically treated with silymarin (100 mg/kg, p.o.), *B. nigra* extract 200 and 400 mg/kg (p.o.), respectively, for 21 days. Group VI received *B. nigra* extract (400 mg/kg, p.o.) alone for 21 days. Groups II, III, IV and V received D-GalN (500 mg/kg, i.p.) on the 22nd day. After 24 h of D-GalN administrations, blood was collected under mild anesthesia. Immediately after blood withdrawal, all the animals were sacrificed by cervical decapitation and tissue samples were collected. Hematological, biochemical, and histological parameters were observed in all groups.

Hematological parameters

Blood samples from the sacrificed animals were analyzed for hematological parameters such as total white blood cells (WBC) count, total lymphocytes (LYM), total monocytes (MONO), total granulocytes (GRAN), lymphocyte percentage (LYM%), monocyte percentage (MONO%), granulocyte percentage (GRAN%), total red blood cells (RBC), hemoglobin (HGB%), hematocrit (HCT%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width

(RDW), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW%) and plateletctrit (PCT) using a BC-2800 Vet AGAPPE Auto hematology analyzer.

Serum biochemistry

The hemolysis free serum sample from the clotted blood was analyzed for the following parameters: blood glucose, cholesterol, total bilirubin, total protein, albumin, globulin, urea, uric acid and creatinine. The enzymes analyzed were serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and γ-glutamyl transferase (γ-GT). The biochemical analysis of serum sample was performed using BAYER–RA-50 semi auto analyzer, BAYER kits and reagents.

Hepatic and renal antioxidant studies

The liver and renal tissues were excised from the rats and homogenized with 10% KCl to make a 10% (w/v) homogenate. The homogenate was centrifuged at 10,000*g* for 15 min in a refrigerated centrifuge at 4°C. The supernatant was used for antioxidant studies such as superoxide dismutase (SOD) (Kakkar et al., 1984), thiobarbutric reactive substances (TBARS) (Ohkawa et al., 1979), glutathione peroxidase (GPx) (Rotruck et al., 1973), reduced glutathione (GSH) (Moren et al., 1979), vitamin C (Oyaizu, 1986), vitamin E (Pilar et al., 1999) and tissue inflammatory markers such as cathepsin D (Sapolsky et al., 1973) and myeloperoxidase (MPO) (Bradley et al., 1982).

Biochemical markers in liver and kidney

The enzyme parameters: ALP, ACP, AST, ALT, γ-GT, and LDH in liver and kidney tissues were measured using Accurex kits, Accurex Biomed Pvt Ltd, Mumbai. Liver and kidney tissue homogenate supernatant (50 µl each) were taken and the activity was measured in a semiautoanalyzer, STAR21plus, USA.

Histological examinations

Liver and kidney were removed from the sacrificed animals, flushed with saline for removal of blood clot and preserved in 10% buffered formalin for histological examination. The paraffin blocks of tissue were microtomed and paraffin sections of thickness 5 µm were obtained. The sections were deparaffinized with alcohol-xylene series and stained with hematoxylin and eosin for examination by light microscopy.

Statistical analysis

The differences among experimental and control groups were determined using SPSS15.0 statistical software. Comparisons were performed by ANOVA test. Resulting data were expressed as mean ± SD ($n = 6$). $p < 0.05$ was considered significant.

Results

Hematological parameters

Table 1 shows the hematological changes in control and different experimental groups. Lymphocytes% and PLT were significantly ($p < 0.001$) lower in (group II) D-GalN intoxicated rats ($p < 0.001$) when compared with control (group I) rats. The levels of lymphocytes% and PLT were increased in group III ($p < 0.001$), group IV and group V ($p < 0.05$) when compared with group II animals. However, there were no statistical significance in group III, group IV and group V when compared with normal control (group I) rats. The granulocytes% was significantly increased ($p < 0.001$) in (group II) D-GalN treated toxic rats ($p < 0.001$) compared to the control rats (group I).The silymarin and *B. nigra* extract treated groups (group III, IV and V) showed values similar to control group but were significantly ($p < 0.05$) lower when compared to group II. Other hematological parameters were not significantly altered among the groups as compared with the normal control rats (Table 1).

Serum biochemistry

A significant increase in serum glucose ($p < 0.001$), cholesterol ($p < 0.001$), total bilirubin ($p < 0.001$), urea ($p < 0.001$), uric acid ($p < 0.001$) and creatinine ($p < 0.001$) were observed in animals treated with D-GalN (group II) as compared the with the normal control group (group I). *B. nigra* extract (200 and 400 mg/kg, p.o) and silymarin (100 mg/kg, p.o) pretreatment for 21 days decreased serum glucose ($p < 0.001$), cholesterol ($p < 0.001$), total bilirubin ($p < 0.001$), urea ($p < 0.001$), uric acid ($p < 0.001$) and creatinine ($p < 0.001$) in groups IV, V and III, respectively, when compared with toxic rats (group II). The activities of SGOT ($p < 0.001$), SGPT ($p < 0.001$), ALP ($p < 0.001$), LDH ($p < 0.001$) and γ-GT ($p < 0.001$) were found to be increased whereas, decreased levels of total protein ($p < 0.001$), and albumin ($p < 0.001$) [globulin-not significant] were observed in animals intoxicated with D-GalN (group II) when compared with normal control group (group I). Pretreatment with *B. nigra* extract (200 and 400 mg/kg, p.o) and silymarin (100 mg/kg, p.o) for 21 days decreased activities of SGOT ($p < 0.001$), SGPT ($p < 0.001$), ALP ($p < 0.001$), LDH ($p < 0.001$) and γ-GT ($p < 0.001$) whereas, the total protein ($p < 0.001$) and albumin ($p < 0.001$) were significantly increased in groups IV, V, and III when compared with group II rats. In *B. nigra* extract alone treated rats (group VI) levels of metabolites and activities of markers enzymes in serum were similar to control group (Table 2).

Tissue enzymic and non-enzymic antioxidants

A significant increase in TBARS ($p < 0.001$) level was observed in liver and kidney tissues of D-GalN intoxicated rats (group II) when compared with control rats (group I), on the other hand the enzymic and non-enzymic antioxidants such as SOD ($p < 0.001$), GPx ($p < 0.001$), GSH ($p < 0.001$), vitamin C ($p < 0.001$) and vitamin E ($p < 0.001$)

Table 1. Effect of methanol extract of *B. nigra* on hemocomponents in control and different experimental groups.

Particulars	Group I	Group II	Group III	Group IV	Group V	Group VI
WBC × 10^9/L	9.91 ± 4.64	6.81 ± 2.69^{a‡}	9.06 ± 0.75^{b‡}	7.95 ± 0.53^{b‡}	8.65 ± 0.55^{b‡}	9.86 ± 3.73^{a‡}
Lymphocytes × 10^9/L	8.1 ± 3.9	4.1 ± 0.88^{a‡}	8.85 ± 1.92^{b‡}	6.88 ± 0.57^{b‡}	7.86 ± 2.22^{b‡}	8.3 ± 3.8^{a‡}
Monocytes × 10^9/L	0.25 ± 0.13	0.21 ± 0.12^{a‡}	0.23 ± 0.12^{b‡}	0.216 ± 0.09^{b‡}	0.23 ± 0.05^{a‡}	0.26 ± 0.07^{a‡}
Granulocytes × 10^9/L	1.41 ± 0.63	2.48 ± 2^{a‡}	1.33 ± 0.28^{b‡}	1.83 ± 0.46^{b‡}	1.56 ± 0.58^{b‡}	1.45 ± 0.18^{a‡}
Lymphocytes (%)	81.2 ± 3.04	54.6 ± 13.71^{a*}	81.86 ± 2.59^{b*}	71.91 ± 2.12^{b‡}	77.78 ± 5.18^{b**}	81.3 ± 3^{a‡}
Monocytes (%)	2.5 ± 0.3	2.7 ± 0.72^{a‡}	2.25 ± 0.17^{b‡}	2.25 ± 0.36^{b‡}	2.3 ± 0.45^{b‡}	2.55 ± 0.22^{a‡}
Granulocytes (%)	16.3 ± 2.87	28.3 ± 13.19^{a*}	15.91 ± 2.49^{a*}	18.45 ± 1.73^{a**}	16.25 ± 1.87^{a*}	16.48 ± 2.79^{a‡}
RBC × 10^{12}/L	7.9 ± 0.65	6.6 ± 2.4^{a‡}	8.45 ± 0.65^{b‡}	7.7 ± 0.46^{b‡}	8.02 ± 0.52^{b‡}	7.92 ± 0.64^{a‡}
Hb (g/L)	13.55 ± 1.06	11.07 ± 4.36^{a‡}	14.71 ± 1.44^{b‡}	12.83 ± 1.62^{b‡}	13.4 ± 0.24^{b‡}	13.83 ± 1.37^{a‡}
Hematocrit (%)	40.18 ± 3.19	35.77 ± 13.42^{a‡}	44.48 ± 4.42^{b‡}	43.31 ± 7.41^{b‡}	43.9 ± 4.38^{b‡}	40.33 ± 3.42^{a‡}
Mean corpuscle volume (fL)	51 ± 2.84	45.81 ± 2.14^{a‡}	52.58 ± 1.6^{b‡}	49.93 ± 5.73^{b‡}	51.71 ± 0.73^{b‡}	51.33 ± 3.009^{a‡}
Mean corpuscular Hb (pg)	17.13 ± 0.95	14 ± 0.84^{a‡}	17.35 ± 0.45^{b‡}	17.1 ± 0.35^{b‡}	17.11 ± 0.49^{b‡}	17.25 ± 1.11^{a‡}
Mean corpuscular Hb concentration (g/L)	30.21 ± 8.26	26.25 ± 1.33^{a‡}	33.2 ± 0.43^{b‡}	32.2 ± 0.55^{b‡}	32.53 ± 0.76^{b‡}	31.05 ± 8.82^{a‡}
RBC distribution width (%)	11.46 ± 1.47	9.2 ± 1.28^{a‡}	11.16 ± 0.46^{b‡}	12.48 ± 2.39^{b‡}	12.95 ± 1.61^{b‡}	11.6 ± 1.34^{a‡}
PLT × 10^9/L	269.5 ± 36.29	198 ± 74.43^{a*}	214.33 ± 26.07^{b†}	228.16 ± 58.95^{b**}	221.83 ± 58.09^{b**}	274.5 ± 43.09^{a‡}
Mean platelet volume (fL)	5.85 ± 0.2	5.77 ± 0.46^{a‡}	6.13 ± 0.35^{b‡}	6 ± 0.28^{b‡}	6 ± 0.15^{b‡}	6.01 ± 0.43^{a‡}
Platelet distribution width (%)	14.88 ± 0.31	13.37 ± 0.64^{a‡}	15.11 ± 0.31^{b‡}	14.95 ± 0.22^{b‡}	14.86 ± 0.18^{b‡}	15.25 ± 0.86^{a‡}
Plateletcrit (%)	0.158 ± 0.02	0.131 ± 0.04^{a‡}	0.131 ± 0.01^{b‡}	0.136 ± 0.03^{b‡}	0.132 ± 0.03^{b‡}	0.163 ± 0.01^{a‡}

Values are expressed as mean ± SD; $n = 6$. Groups were treated as mentioned in "Materials and methods".
$^*p < 0.001$, $^{**}p < 0.01$, $^\dagger p < 0.05$, ‡not significant.
[a]as compared with group I.
[b]as compared with group II.

Table 2. Effect of methanol extract of *B. nigra* on serum biochemical components in control and different experimental groups.

Particulars	Group I	Group II	Group III	Group IV	Group V	Group VI
Blood glucose (mg/dL)	73 ± 3.4	86.83 ± 1.47^{a*}	75 ± 1.89^{b*}	69.16 ± 1.16^{b*}	69.66 ± 1.63^{b*}	72.5 ± 2.42^{a‡}
Cholesterol (mg/dL)	58.66 ± 1.36	127.16 ± 1.47^{a*}	61.33 ± 1.63^{b*}	78.33 ± 1.21^{b*}	71.83 ± 1.16^{ba*}	58.5 ± 1.04^{a‡}
Total protein (g/dL)	8.03 ± 0.16	5.41 ± 0.19^{a*}	7.36 ± 0.13^{b*}	6.641 ± 0.19^{b*}	7.53 ± 0.15^{b*}	7.9 ± 0.41^{a‡}
Albumin (g/dL)	5.38 ± 0.22	2.9 ± 0.17^{a*}	4.68 ± 0.17^{b*}	3.95 ± 0.25^{b*}	4.58 ± 0.14^{b*}	5.45 ± 0.12^{a‡}
Globulin (g/dL)	2.65 ± 0.18	2.51 ± 0.07^{a‡}	2.68 ± 0.11^{b‡}	2.69 ± 0.12^{b‡}	2.95 ± 0.13^{b*}	2.46 ± 0.27^{a‡}
Bilirubin (mg/dL)	0.336 ± 0.02	2.8 ± 0.08^{a*}	0.741 ± 0.04^{b*}	1.066 ± 0.08	0.85 ± 0.05	0.338 ± 0.02^{a‡}
Urea (mg/dL)	36.5 ± 1.04	84.5 ± 1.87^{a*}	47.33 ± 0.81^{b*}	51.5 ± 1.04^{b*}	48.16 ± 0.75^{b*}	36.83 ± 1.16^{a‡}
Uric acid (mg/dL)	0.93 ± 0.1	3.53 ± 0.33^{a*}	1.26 ± 0.12^{b*}	2.33 ± 0.1^{b*}	1.9 ± 0.06^{b*}	0.91 ± 0.07^{a‡}
Creatinine (mg/dL)	0.525 ± 0.02	1.146 ± 0.07^{a*}	0.633 ± 0.05^{b*}	0.866 ± 0.05^{b*}	0.733 ± 0.05^{b*}	0.525 ± 0.02^{a‡}
SGOT (IU/L)	105.16 ± 2.99	203.33 ± 5.27^{a*}	118.5 ± 1.04^{b*}	142.16 ± 2.78^{b*}	123.5 ± 1.87^{b*}	105.5 ± 2.5^{a‡}
SGPT (IU/L)	65.33 ± 0.81	119.33 ± 2.73^{a*}	71.66 ± 1.63^{b*}	90.5 ± 1.04^{b*}	80.66 ± 1.36^{b*}	65.83 ± 0.75^{a‡}
ALP (IU/L)	192.66 ± 1.36	374 ± 7.12^{a*}	240.33 ± 1.75^{b*}	310.33 ± 4.71^{b*}	272.33 ± 3.77^{b*}	193.16 ± 1.16^{a‡}
LDH (IU/L)	419.5 ± 11.67	737.83 ± 4.87^{a*}	503 ± 5.09^{b*}	673.66 ± 4.8^{b*}	624.66 ± 5.46^{b*}	420 ± 10.52^{a‡}
GGT (IU/L)	5.23 ± 0.16	9.273 ± 0.43^{a*}	7.55 ± 0.28^{b*}	8.11 ± 0.11^{b*}	7.7 ± 0.08^{b*}	5.28 ± 0.13^{a‡}

Values are expressed as mean ± SD; $n = 6$. Groups were treated as mentioned in "Materials and methods."
$^*p < 0.001$, ‡not significant.
[a]as compared with group I.
[b]as compared with group II.

were decreased. Pretreatment with *B. nigra* extract (group IV and V) and silymarin (group III) significantly decreased TBARS and increased the activities of SOD ($p < 0.05$) and GPx ($p < 0.05$) in liver and kidney tissues when compared with group II rats, the non-enzymic antioxidant levels [GSH ($p < 0.05$), vitamin C ($p < 0.05$) and vitamin E ($p < 0.05$)] were also increased when compared with D-GalN intoxicated rats (group II). In *B. nigra* extract alone treated rats (group VI), levels of TBARS, GSH, vitamin C and vitamin E, the activities of SOD and GPx in liver and kidney were similar to that of control group (Tables 3 and 4).

Inflammatory markers

The changes in the activities of ACP, MPO and cathepsin D in liver and kidney of the various experimental groups are shown in Tables 5 and 6. A marked increase in activities of ACP ($p < 0.001$), MPO ($p < 0.001$) and cathepsin D ($p < 0.001$) in liver and kidney tissues were observed in D-GalN intoxicated rats (group II), whereas pretreatment with *B. nigra* (group IV and V) and silymarin (group III) decreased the activities significantly ($p < 0.001$). In *B. nigra* extract alone treated rats (group VI) activities of these enzymes were found to be similar as that of control rats (Tables 5 and 6).

Table 3. Effect of methanol extract of *B. nigra* on lipid peroxidation and antioxidant components in liver of control and experimental groups.

Particulars	Lipid peroxidation	SOD	Vitamin E	Vitamin C	GPx	GSH
Group I	0.23 ± 0.041	1.13 ± 0.11	2.51 ± 0.15	0.451 ± 0.04	41.33 ± 2.3	3.19 ± 0.24
Group II	1.181 ± 0.18[a*]	0.51 ± 0.08[a*]	1.06 ± 0.07[a*]	0.285 ± 0.02[a*]	17.53 ± 1.23[a*]	1.115 ± 0.14[a*]
Group III	0.296 ± 0.02[b*]	0.818 ± 0.02[b*]	2.153 ± 0.14[b*]	0.401 ± 0.03[b*]	36.29 ± 2.57[b*]	2.83 ± 0.08[b*]
Group IV	0.409 ± 0.01[b*]	0.575 ± 0.01[b†]	1.345 ± 0.12[b**]	0.332 ± 0.01[b†]	20.86 ± 0.7[b**]	2.265 ± 0.14[b*]
Group V	0.331 ± 0.03[b*]	0.725 ± 0.11[b**]	1.661 ± 0.05[b*]	0.376 ± 0.01[b*]	24.41 ± 1.1[b*]	2.683 ± 0.21[b*]
Group VI	0.226 ± 0.04[a†]	1.133 ± 0.1[a†]	2.485 ± 0.19[a†]	0.468 ± 0.03[a†]	41.16 ± 2.26[a†]	3.186 ± 0.25[a†]

Values are expressed as mean ± SD; $n = 6$. Units are expressed as: nmoles of TBARS formed/mg tissue for lipid peroxidation; U/min/mg protein (One unit – 50% inhibition of NBT reduction in 1 min) for SOD; mg/g tissue for vitamin E, vitamin C & GSH; nmoles of GSH oxidized/min/mg protein for GPx. Groups were treated as mentioned in "Materials and methods."

*$p < 0.001$, **$p < 0.01$, †not significant.

[a]as compared with group I

[b]as compared with group II.

Table 4. Effect of methanol extract of *B. nigra* on lipid peroxidation and antioxidant components in kidney of D-galactosamine intoxicated rats.

Particulars	Lipid peroxidation	SOD	Vitamin E	Vitamin C	GPx	GSH
Group I	0.026 ± 0.005	1.341 ± 0.06	0.453 ± 0.05	2.28 ± 0.11	41.56 ± 2.76	3.053 ± 0.11
Group II	0.128 ± 0.01[a*]	0.22 ± 0.02[a*]	0.215 ± 0.008[a*]	0.306 ± 0.02[a*]	11.12 ± 0.45[a*]	0.978 ± 0.11[a*]
Group III	0.038 ± 0.007[b*]	1.215 ± 0.11[b*]	0.363 ± 0.01[b*]	1.893 ± 0.03[b*]	40.77 ± 1.87[b*]	2.5 ± 0.19[b*]
Group IV	0.058 ± 0.007[b*]	0.708 ± 0.02[b*]	0.241 ± 0.01[b†]	0.776 ± 0.02[b*]	27.28 ± 1.14[b*]	1.301 ± 0.06[b*]
Group V	0.041 ± 0.007[b*]	0.883 ± 0.04[b*]	0.286 ± 0.01[b*]	0.895 ± 0.02[b*]	35.56 ± 2.94[b*]	1.518 ± 0.12[b*]
Group VI	0.028 ± 0.004[a†]	1.358 ± 0.04[a†]	0.456 ± 0.05[a†]	2.296 ± 0.09[a†]	41.39 ± 2.95[a†]	3.07 ± 0.09[a†]

Values are expressed as mean ± SD; $n = 6$. Units was expressed as: nmoles of TBARS formed/mg tissue for lipid peroxidation; U/min/mg protein (One unit – 50% inhibition of NBT reduction in 1 min) for SOD; mg/g tissue for vitamin E, vitamin C & GSH; nmoles of GSH oxidized/min/mg protein for GPx. Groups were treated as mentioned in materials and methods.

*$p < 0.001$, †not significant.

[a]as compared with group I.

[b]as compared with group II.

Table 5. Effect of methanol extract of *B. nigra* on inflammatory markers in liver of different experimental groups.

Particulars	Acid Phosphatase	Cathepsin D	Myeloperoxidase
Group I	0.016 ± 0.004	0.246 ± 0.07	2.996 ± 1.37
Group II	0.061 ± 0.007	1.178 ± 0.16[a*]	8.406 ± 1.7[a*]
Group III	0.015 ± 0.005	0.318 ± 0.007[b*]	3.39 ± 0.18[b*]
Group IV	0.04 ± 0.006	0.703 ± 0.09[b*]	6.866 ± 0.84[b†]
Group V	0.018 ± 0.007	0.576 ± 0.03[b*]	4.878 ± 0.43[b*]
Group VI	0.014 ± 0.005[a†]	0.247 ± 0.07[a†]	3.005 ± 1.36[a†]

Values are expressed as mean ± SD; $n = 6$. Activities are expressed as: µmoles of phosphate liberated/min/mg protein for acid phosphatase; µmoles of tyrosine released/min/mg protein for cathepsin D; nmoles of peroxide degraded/min/mg protein for myeloperoxidase. Groups were treated as mentioned in "Materials and methods."

*$p < 0.001$, †not significant.

[a]as compared with group I.

[b]as compared with group II.

Table 6. Effect of methanol extract of *B. nigra* on inflammatory markers in kidney of D-galactosamine intoxicated rats.

Particulars	Acid Phosphatase	Cathepsin D	Myeloperoxidase
Group I	0.013 ± 0.005	0.1 ± 0.02	14.61 ± 3.13
Group II	0.066 ± 0.008[a*]	0.41 ± 0.02[a*]	33.77 ± 10.98[a*]
Group III	0.018 ± 0.007[b*]	0.123 ± 0.01[b*]	16.73 ± 4.4[b*]
Group IV	0.038 ± 0.007[b*]	0.39 ± 0.01[a†]	25.08 ± 6.64[a†]
Group V	0.021 ± 0.004[b*]	0.288 ± 0.02[b*]	18.36 ± 2.18[b*]
Group VI	0.011 ± 0.004[a†]	0.11 ± 0.03[b†]	14.78 ± 3.05[b†]

Values are expressed as mean ± SD; $n = 6$. Activities are expressed as: µmoles of phosphate liberated/min/mg protein for acid phosphatase; µmoles of tyrosine released/min/mg protein for cathepsin D; nmoles of peroxide degraded/min/mg protein for myeloperoxidase. Groups were treated as mentioned in "Materials and methods."

*$p < 0.001$, †not significant.

[a]as compared with group I.

[b]as compared with group II.

Tissue enzymes

In D-GalN intoxicated rats (group II), the activities of marker enzymes, viz., ALP ($p < 0.001$), AST ($p < 0.001$), ALT ($p < 0.001$), LDH ($p < 0.001$) and γ-GT (($p < 0.001$) were found to be significantly decreased in liver and kidney tissues when compared with control (group I) rats (Tables 7 and 8). Whereas, in *B. nigra* extract (group IV and V) and silymarin (group III) pretreated rats, activities of these enzymes, in liver and kidney, were found to be significantly ($p < 0.001$) improved when compared with group II rats. In *B. nigra* extract alone treated rats (group VI), the activities of marker enzymes were found to be similar with that of control rats.

Histopathological observations of liver and kidney

Histology of the liver and kidney sections of normal control animals treated with saline vehicle alone (group I) shows normal architecture (Figures 1A and 2A). Animals treated with D-GalN alone (group II) show severe toxicity characterized by scattered inflammation across

Table 7. Activities of some marker enzymes in liver of different experimental groups.

Particulars	Alkaline phosphatase	Aspartate aminotransferase	Alanine aminotransferase	Lactate dehydrogenase	γ-Glutamyl transferase
Group I	1.06 ± 0.06	0.415 ± 0.02	1.806 ± 0.14	0.196 ± 0.02	0.618 ± 0.03
Group II	0.043 ± 0.008[a*]	0.051 ± 0.03[a*]	0.22 ± 0.15[a*]	0.041 ± 0.03[a*]	0.075 ± 0.01[a*]
Group III	0.908 ± 0.05[b*]	0.388 ± 0.02[b*]	1.54 ± 0.22[b*]	0.17 ± 0.02[b*]	0.571 ± 0.03[b*]
Group IV	0.526 ± 0.04[b*]	0.25 ± 0.02[b*]	0.913 ± 0.08[b*]	0.071 ± 0.01[b‡]	0.343 ± 0.14[b*]
Group V	0.815 ± 0.06[b*]	0.365 ± 0.09[b*]	1.206 ± 0.07[b*]	0.111 ± 0.03[b*]	0.54 ± 0.09[b*]
Group VI	1.051 ± 0.063377[a‡]	0.431 ± 0.03[a‡]	1.79 ± 0.14[a‡]	0.195 ± 0.02[a‡]	0.615 ± 0.03[a‡]

Values are expressed as mean ± SD; $n = 6$. Activities was expressed as:μmoles of phosphate liberated/min/mg protein for alkaline phosphatase: μmoles of malate liberated/min/mg protein for aspartate aminotransferase; μmoles of lactate liberated/min/mg protein for alanine aminotransferase; μmoles of pyruvate consumed/min/mg protein for lactate dehydrogenase; μmoles of 5-amino-2-nitrobenzoate liberated/min/mg protein for γ-glutamyl transferase. Groups were treated as mentioned in "Materials and methods."

*$p < 0.001$, ‡not significant.

[a]as compared with group I.

[b]as compared with group II.

Table 8. Effect of *B. nigra* on activities of some marker enzymes in kidney of different experimental groups.

Particulars	Alkaline phosphatase	Aspartate aminotransferase	Alanine aminotransferase	Lactate dehydrogenase	γ-Glutamyl transferase
Group I	0.833 ± 0.08	0.431 ± 0.03	0.580 ± 0.09	1.126 ± 0.13	0.15 ± 0.02
Group II	0.073 ± 0.008[a*]	0.118 ± 0.02[a*]	0.125 ± 0.008[a*]	0.22 ± 0.08[a*]	0.026 ± 0.008[a*]
Group III	0.71 ± 0.02[b*]	0.395 ± 0.03[b*]	0.519 ± 0.03[b*]	0.911 ± 0.05[b*]	0.126 ± 0.03[b*]
Group IV	0.41 ± 0.08[b*]	0.218 ± 0.08[b‡]	0.322 ± 0.04[b*]	0.536 ± 0.1[b*]	0.078 ± 0.03[b‡]
Group V	0.618 ± 0.09[b*]	0.34 ± 0.08[b*]	0.451 ± 0.007[b*]	0.815 ± 0.08[b*]	0.095 ± 0.03[b*]
Group VI	0.826 ± 0.08[a‡]	0.423 ± 0.03[a‡]	0.584 ± 0.08[a‡]	1.13 ± 0.13[a‡]	0.148 ± 0.02[a‡]

Values are expressed as mean ± SD; $n = 6$. Activities was expressed as: μmoles of phosphate liberated/min/mg protein for alkaline phosphatase; μmoles of malate liberated/min/mg protein for aspartate aminotransferase; μmoles of lactate liberated/min/mg protein for alanine aminotransferase; μmoles of pyruvate consumed/min/mg protein for lactate dehydrogenase; μmoles of 5-amino-2-nitrobenzoate liberated/min/mg protein for γ-glutamyl transferase. Groups were treated as mentioned in "Materials and methods."

*$p < 0.001$, ‡not significant.

[a]as compared with group I.

[b]as compared with group II.

liver parenchyma, sinusoidal congestion, vacuolar degeneration and necrosis of hepatocytes in liver sections (Figure 1B) tubular epithelial cell degeneration, necrosis and perivascular mononuclear cell infiltration in kidney sections (Figure 2B). *B. nigra* extract pretreatment (group IV and V) appeared to alleviate D-GalN toxicity as revealed by reduction in degeneration of hepatocytes of liver sections and tubular epithelial cells of kidney sections when compared with group II rats (Figures 1D and 1E; 2D and 2E). Rats pretreated with silymarin (group III) exhibited protection against D-GalN induced degenerative changes in the liver and kidney (Figures 1C and 2C). Histology of the liver and kidney of rats pretreated with *B. nigra* extract alone (group VI) shows normal architecture as control animals (group I) (Figures 1F and 2F). We did not observe any differences in liver or kidney histopathological (biochemical analysis also) sections obtained in male and female of different experimental rats.

Discussion

Study of herbal drugs is gaining more attention due to their ameliorating effect on acute and chronic disease conditions. The plant extracts have been used in traditional medicines for centuries, since they act as a source of antioxidants and efficient pharmacophores. In the present investigation, protective effects of the *B. nigra* methanol extract were studied in D-GalN induced liver and kidney damages. D-GalN induced liver damage in rats is a model system which is recognized to be much similar to viral hepatitis in humans from both morphological and physiological point of view (Keppler et al., 1968). D-GalN causes hepatic injury with spotty necrosis of hepatic parenchyma and marked portal and parenchymal infiltration (Keppler & Decker, 1969). D-GalN also causes depletion of uridine diphosphate (UDP) by increasing the formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis leading to cell membrane deterioration (Decker et al., 1973; EI-Mofty et al., 1975).

Javle et al. (1998) reported that D-GalN-induced liver injury is associated with alteration in renal hemodynamics thus altering its function. Recently the term hepatorenal syndrome (HRS) has been introduced to define the development of renal failure in the absence of its clinical, anatomical and pathological causes. Classically, HRS is associated with the end-stage liver cirrhosis and it has been observed that renal failure occurs with this liver disease in about 50% of patients. Anand et al. (2002) reported that D-GalN intoxication causes liver injury followed by renal impairment which is manifested by the decrease in renal blood flow and creatinine clearance.

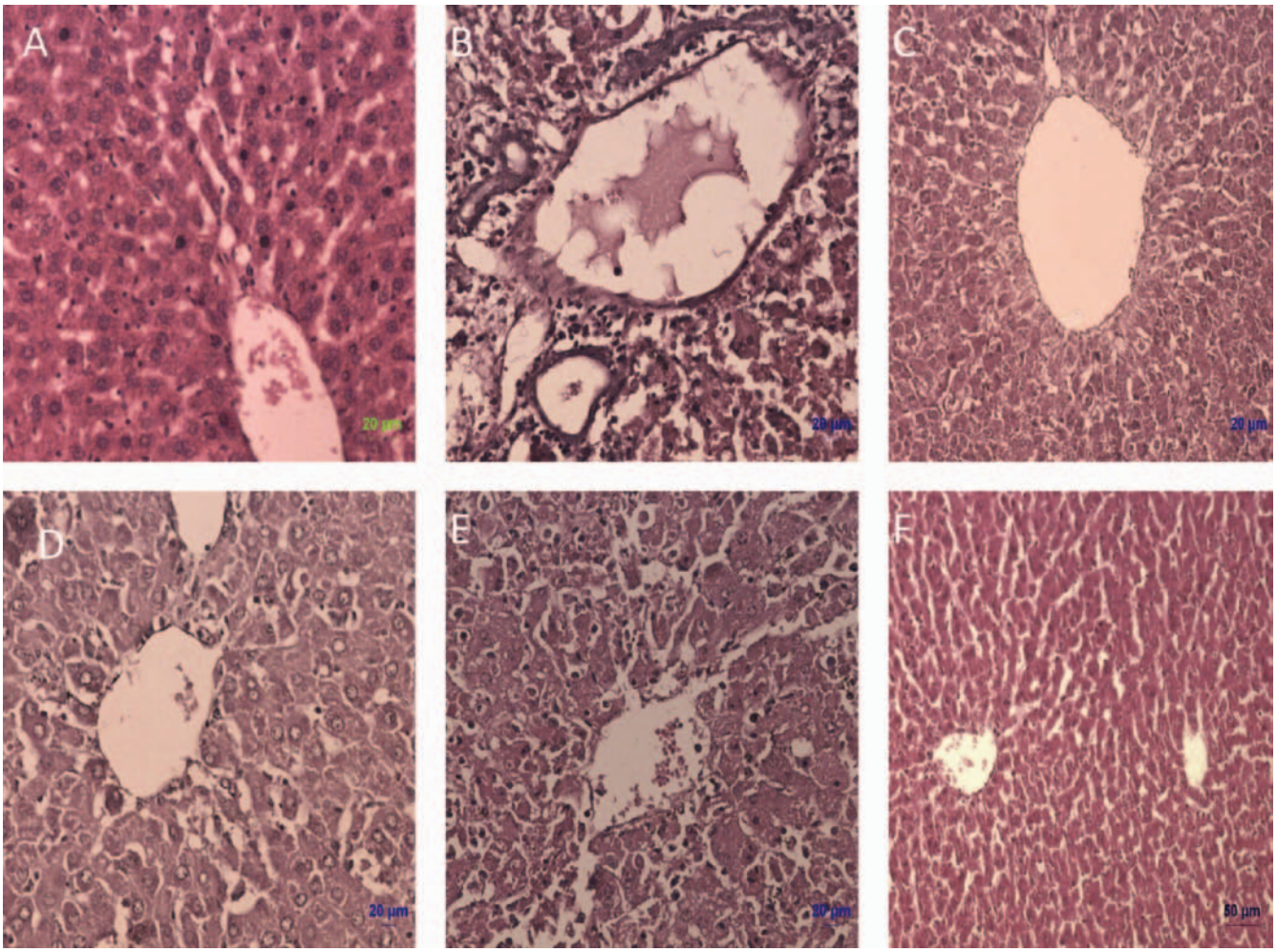

Figure 1. Histological observation in liver sections (Hematoxylin and Eosin staining) of different experimental groups. (A) control, (B) toxic control (D-galactosamine, 500 mg/kg, i.p.), (C) standard (silymarin, 100 mg/kg), (D) *B. nigra* extract (200 mg/kg, p.o.) + D-galactosamine treated, (E) *B. nigra* extract (400 mg/kg, p.o.) + D-galactosamine treated, and (F) *B. nigra* extract (400 mg/kg, p.o.) alone treated.

Renal dysfunction due to D-GalN administration was also manifested by the increase in serum urea and creatinine levels and kidney TBARS and MPO activity together with degenerative changes in kidney histology (Tunc et al., 2010).

D-GalN administration in rats disrupts the membrane permeability of the plasma membrane causing leakage of the enzymes and metabolites from the cell, which leads to elevation in activities of serum enzyme and metabolites (Mitra et al., 2000). Elevated serum enzymes and metabolites are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver (Drotman & Lawhorn, 1978). As a result of the cellular leakage, activities of these enzymes in liver tissue were also decreased. In our study, significant increase in the activities of serum enzymes, viz., SGOT, SGPT, ALP, LDH and γ-GT, the levels of metabolites such as total bilirubin, urea, uric acid and creatinine were also increased in D-GalN intoxicated rats. We also observed decreased levels of the total protein and albumin in D-GalN intoxicated rats with concomitant decrease in activities of ALP, AST, ALT, γ-GT and LDH in liver and kidney. Pretreatment with *B. nigra* extract and silymarin reversed these biochemical alterations, indicating that *B. nigra* extract has protective effects against D-GalN-induced liver and kidney injury.

A detailed mechanism of the hepato-and nephrotoxic effects induced by D-GalN has not been found, but the toxicity might be mediated through the immune system or oxidative stress. Vitamin E and vitamin C are naturally occurring free radical scavengers (Yu, 1994). Both vitamin E and vitamin C are known to be decreased in liver diseases maybe due their increased utilization for scavenging oxygen-derived radicals (Johnson et al., 1987). Glutathione is an important endogenous antioxidant system that is found in particularly high concentration in liver and is known to have key functions in protective processes. The GSH becomes readily oxidized (GSSG) upon interacting with free radicals. Excessive production of radicals results in oxidative stress, which leads to disruption of the macromolecules, e.g. lipids and can induce lipid peroxidation (Sinclair et al., 1991). In present investigation, there was a significant decrease in the activities of tissue enzymic

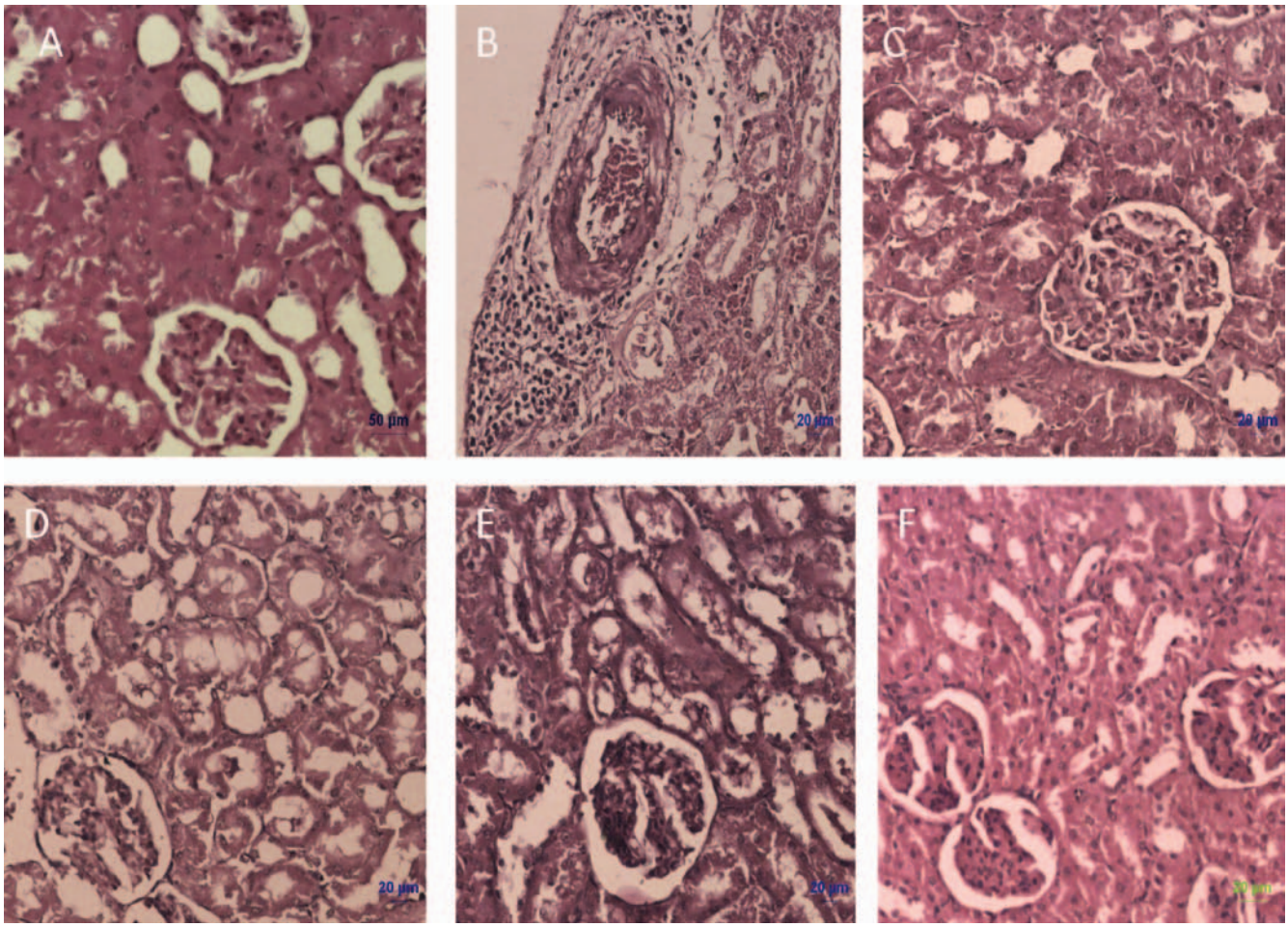

Figure 2. Histological observation in renal sections (Hematoxylin and Eosin staining) of different experimental groups. (A) control, (B) toxic control (D-galactosamine, 500 mg/kg, i.p.), (C) standard (Silymarin, 100mg/kg), (D) *B. nigra* extract (200 mg/kg, p.o.) + D-galactosamine treated, (E) *B. nigra* extract (400 mg/kg, p.o.) + D-galactosamine treated and (F) *B. nigra* extract (400 mg/kg, p.o.) alone treated.

antioxidants such as SOD and GPx and increase in the level of TBARS with a concomitant decrease in levels of nonenzymic antioxidants such as vitamin E, vitamin C and GSH in D-GalN intoxicated rats (group II) which indicate severity of oxidative stress induced as a result of administration of D-GalN. Considerable increase in the activities of antioxidant enzymes, decrease in the level of TBARS, and increased levels of GSH, vitamin C, and vitamin E in the *B. nigra* extract pretreated rats (group IV and V) clearly indicate that *B. nigra* extract possesses *in vivo* antioxidant effect. We have shown that *B. nigra* extract possesses *in vitro* antioxidant activity (Rajamurugan et al., 2011).

Lysosomal enzyme activities in inflammatory exudates serve as a good marker to assess the intensity of inflammation in experimental groups. Hydrolytic enzymes are released by the rupture of the lysosomal membrane, which in turn initiates the synthesis of inflammatory mediators. Drugs capable of stabilizing the lysosomal membrane can reduce the inflammation (Azza & Mohamed, 1995). An important mechanism of anti-inflammatory activity is due to the membrane stability–modulating effect (De et al., 1994). Cathepsin D has been found to play a role in the intracellular degradation of exogenous and endogenous proteins (Igdoura et al., 1995). The proteolytic activity of cathepsin D is increased during various pathogenic processes leading to injury of lysosomes (Eiki et al., 1991). Extensive infiltration of leukocytes leads to an increase in lysosomal hydrolases (Anderson, 1970). The marked decrease in liver and kidney tissue ACP, cathepsin D and MPO activities in extract treated groups (groups IV and V) indicates that the *B. nigra* may have membrane stabilization effect. Many sesquiterpenes are found to possess anti-inflammatory activity (Hall et al., 1979). Thus the anti-inflammatory activity may be due to the terpenoids that may be present in the *B. nigra* extract. *B. nigra* seed extract has shown, one of the excellent sources of terpenoids (Jakupovic et al., 1986; Kakali et al., 1997). Our previous findings show that *B. nigra* leaf also contains terpenoids (Rajamurugan et al., 2011). The present study suggests that methanol extract of *B. nigra* may also possess anti-inflammatory activity.

D-GalN is reported to produce intensive inflammatory infiltration in the liver and kidney parenchyma and peripheral area (Keppler et al., 1968; Tunc et al., 2010). In our study, D-GalN administration shows severe hepato-and nephrotoxic effects with heavy infiltration of

inflammatory cells around the parenchyma of liver and kidney. Silymarin is a well known hepatoprotective drug in wide usage; hence, the hepatoprotective effect of *B. nigra* was compared with that of silymarin (Pradhan & Girish, 2006). Pretreatment with *B. nigra* leaf extract and silymarin protected the rat liver and kidney from D-GalN-induced histopathological changes. The hepatoprotective effect might have been contributed by hepatoprotectants such as (*Z*, *Z*)- 9,12-octadecadienoic acid (linoleic acid) and α-amyrin (triterpenoid) and/or also due to presence of various antioxidant present in the extract, which was reported from our recent investigation (Rajamurugan et al., 2011).

Conclusions

On the basis of the results obtained in the present study, it is evident that the methanol extract of *B. nigra* leaves exhibit protective effect against D-GalN-induced hepatic and renal injury. Biochemical observations were supported by histological examinations of liver and kidney. The group treated with *B. nigra* extract alone proved that the extract is non-toxic and is safe. Based on the antioxidant and anti-inflammatory effects of extract from *B. nigra* leaves it may be suggested as a remedy in treatment of hepatic and renal injury.

Acknowledgments

One of the authors (R.R) is very grateful to the Director, additional director, research officers, staff in department of pharmacology and biochemistry, Siddah Central Research Institute, Chennai, India and Prof. S. P. Thyagarajan, Pro-chancellor (Research), Sri Ramachandra University, Chennai, India. He also acknowledges Head and the department of veterinary pathology, Madras Veterinary College, Chennai.

Declaration of interest

The authors declare no conflicts of interest.

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