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Pharmacological credence of the folklore use of *Bauhinia malabarica* in the management of jaundiceK. Thenmozhi^a, N. Anusuya^b, M. Ajmal Ali^{c,*}, S. Jamuna^a, K. Karthika^a, A. Venkatachalapathi^a, F.M. Al-Hemaid^c, M.A. Farah^d, S. Paulsamy^a^a Department of Botany, Kongunadu Arts and Science College, Coimbatore 641029, Tamil Nadu, India^b Department of Botany, Alagappa University, Karaikudi 630 003, Tamil Nadu, India^c Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia^d Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

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ABSTRACT

The information on the hepatoprotective effect of *Bauhinia malabarica* Roxb. (Family Leguminosae) used in the folkloric medical practice in Malabar coast and Walayar valley of southern India for the treatment of liver related disorders is completely unknown. Hence, the efficacy of the aqueous methanolic extract of stem bark of *B. malabarica* (AqMeOH-Ba) was evaluated for liver function serum biochemical markers along with the antioxidant markers in liver tissues of Wistar albino rats. The biochemical observations as well as the histopathological examination of liver sections manifested considerable hepatoprotective activity of *B. malabarica* stem bark, and thus validated the folkloric claim.

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1. Introduction

The genus *Bauhinia* Roxb. (Leguminosae) mainly found in tropical areas, comprises more than 300 species. The root, stem bark, leaves and flowers of the genus *Bauhinia* are medicinally valuable. It is commonly use in various indigenous systems of medicine to treat diabetes, ulcers, tumors, skin diseases, inflammations, wounds and liver disorders (Thenmozhi et al., 2013). The biological studies have previously been demonstrated the therapeutic properties of several species of the genus *Bauhinia* (Ahmed et al., 2012; Thenmozhi et al., 2013; Atram, 2015).

Bauhinia malabarica Roxb. is a deciduous tree of moderate sized, distributed mainly in the sub-Himalayan tracts and southern parts of India. The young shoots of *B. malabarica* are edible, and is being commonly prescribed to treat cough, gout, glandular swellings and goiter, haemorrhage, leprosy, menorrhagia, scrofula, urinary

disorders, wasting diseases, worm infestations and wounds (Ahmed et al., 2012) and for liver disorders (Venkatachalapathi et al., 2015) by the folkloric medical practitioner in Malabar coast and Walayar valley of southern India. The pharmacological information on the hepatoprotective effect of *B. malabarica* is completely unknown; hence, the present study attempted to evaluate the hepatoprotective effect of *B. malabarica* stem bark employing CCl₄ intoxicated rat model.

2. Materials and methods

2.1. Plant material

The stem bark of *B. malabarica* was collected from Siruvani hills of Coimbatore, Tamil Nadu, India. The taxonomic identification of the plant material was confirmed from Botanical Survey of India, Southern circle, Coimbatore (Vide No: BSI/SC/5/23/08-09/Tech.-1718). The stem bark materials were washed, shade dried, and powdered using Wiley grinding Mill.

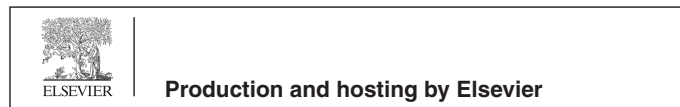
2.2. Sample preparation

The stem bark powder was extracted with aqueous methanol (50%) using soxhlet apparatus. The extract was concentrated using rotary vacuum evaporator (Yamato rotary vacuum evaporator,

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Japan). The extract/test compound (AqMeOH-Ba) was subsequently frozen (Operon, Korea), lyophilized (Virtis, USA) and stored at -20°C until used for experiments.

2.3. Chemicals

Carbon Tetrachloride (CCl_4) was obtained from Sigma- Aldrich Chemicals, Mumbai. Silymarin was purchased from Micro labs, India. All other chemicals and reagents were of analytical grade obtained from Merck, HiMedia and SD Fine Chemicals, Mumbai.

2.4. Experimental animals

Male Swiss albino mice (25–30 g) and male Wistar albino rats (150–250 g) were obtained from the Small Animals Breeding Station, Mannuthy, Kerala, India. All the animals were housed in poly-acrylic cages, and maintained under standard environmental conditions (14 h dark/10 h light cycles, $25 \pm 2^{\circ}\text{C}$ temperature, 35–60% humidity, air ventilation), were fed with a balanced commercial diet (M/S Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were acclimatized to laboratory conditions for 15 days before the experiment. All the animal experiments performed were sanctioned by the Institutional Animal Ethical Committee (IAEC) of Kovai Medical Centre and Hospital (KMCH), Tamil Nadu, India (Approval no: KMCRET/PH.D/15/2008) constituted under the guidelines of CPCSEA (Committee for the Purpose of the Control and Supervision of Experiments on Animals).

2.5. Behavioral and toxicological effects

Swiss albino mice (25–30 g) fasted for 12 h were categorized into six groups of six animals each. The first group was served as control (treated with normal water). The groups two to six were orally treated with the single graded dose (1000, 2000, 3000, 4000, 5000 mg/kg b.w. respectively) of AqMeOH-Ba. After administration, the effect on mortality, clinical signs and gross behavior were observed at 0 h, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h and 72 h.

2.6. Hepatoprotective activity

2.6.1. CCl_4 induced hepatotoxicity

The Wistar albino rats were categorized into five groups of six animals each. Group 1 served as normal control while group 2 served as toxic control. Groups 3 and 4 were orally treated with AqMeOH-Ba at the dose of 250 mg/kg b.w. and 500 mg/kg b.w. respectively, once daily for 15 days. Animals of group 5 (standard group) received 25 mg/kg b.w. silymarin (a widely used herbal drug from *Silibum marianum*) daily for 15 days. On days 7 and 14, all animals except group 1 were administered orally with 1.5 mL/kg b.w. of CCl_4 /olive oil mixture (1: 3 v/v), 1 h after their respective assigned treatments. All the drug preparations were fed orally to rats of respective groups by gastric intubation. Soon after the last dosage, all the animals were fasted but had free access of water for 24 h. The blood samples were drawn from all groups by cardiac puncture using sterile syringes, allowed to clot for 20–30 min, and then centrifuged in a refrigerated centrifuge (4°C) for 10 min at 3000 rpm. The serum samples were stored at -20°C for the biochemical tests. The animals were then euthanized by cervical dislocation. The liver samples were carefully excised, perfused with ice cold normal saline, patted dry with blotting paper, weighed, cut into small pieces, and then homogenized in 0.1 M phosphate buffer, pH 7.0 and centrifuged at 10,000 rpm for 10 min at 4°C . The collected supernatant were used for enzyme assays.

2.6.2. Assessment of liver function

The serum biochemical parameters viz., total protein, albumin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), acid phosphatase (ACP), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, high density lipoproteins (HDL), low density lipoproteins (LDL), total cholesterol (TC) and triglycerides (TG) were assessed in an autoanalyzer (MISPA EXCEL, Mispa biosystems chemistry analyzer) using diagnostic kits (Agappe diagnostics, India). A 10% homogenate of liver tissue was used for the analysis of lipid peroxidation (LPO) (Niehius and Samuelsson, 1968), superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck et al., 1973), glutathione-S-transferase (GST) (Habig et al., 1974), reduced glutathione (Moron et al., 1979) and vitamin C (Omeye et al., 1979).

2.6.3. Histological studies

The liver slices fixed for 48 h in 10% formalsaline were processed for paraffin embedding following the standard microtechnique (Galigher and Kozloff, 1971; Rao and Mishra, 1998). Sections of livers were stained and evaluated for histopathological changes under compound microscope (Carl Zeiss, Germany).

2.7. Statistical analysis

The results were presented as mean \pm SEM ($n = 6$). The statistical analysis was performed using analysis of variance (ANOVA), followed by Tukey's multiple comparison range test (Graph Pad Prism 4.0 software). $P < 0.05$ was chosen as the criterion for the statistical significance.

3. Results and discussion

3.1. Acute toxicity

The hepatoprotective potential of the AqMeOH-Ba was evaluated using CCl_4 induced liver damage in rats. The acute oral administration of test compound AqMeOH-Ba revealed the non-toxic effect, it did not induce any drug - related toxicity even at the highest dose (5000 mg/kg) as compared to the control; hence, two doses of AqMeOH-Ba i.e., 250 and 500 mg/kg were selected for pharmacological studies. CCl_4 is a well-known compound that causes hepatotoxicity by an acute exposor, and it induces cirrhosis (Basu, 2003), ultimately establish a condition of oxidative stress in which the defense capabilities of cells against ROS (e.g. superoxide anion, hydrogen peroxide and the hydroxyl radical) becomes insufficient (Rikans et al., 1994; Weber et al., 2003). Since free radicals play a pivotal role in CCl_4 -induced liver damage; therefore, it is logical that bioactive entities that neutralize free radicals might possess hepatoprotective effect (Zhu et al., 2004).

3.2. Serum biochemical profile

The serum liver function markers like SGOT, SGPT, ALP, ACP, total and direct bilirubin, total protein and albumin were significantly elevated ($P < 0.001$) in CCl_4 induced hepatotoxic rats (Table 1). Administration of AqMeOH-Ba attenuated the elevated levels to normal level. The potency with which the high dose (500 mg/kg b.w.) of AqMeOH-Ba acted upon the markers was more or less equivalent to silymarin (50 mg/kg b.w.). The total protein and albumin content in serum in the present study registered a significant hike in CCl_4 intoxicated rats, which was retrieved to near normalcy in the AqMeOH-Ba treated groups indicating the improvement of the functional integrity of the liver cells. Though enhancement in serum protein levels and albumin in CCl_4 - treated

Table 1
Effect of aqueous methanolic extract of *B. malabarica* stem bark on serum biochemical markers in CCl₄ intoxicated rats.

Parameter	Treatment group				
	Control	CCl ₄ treated	CCl ₄ + extract (250 mg/kg b.w.)	CCl ₄ + extract (500 mg/kg b.w.)	CCl ₄ + silymarin (25 mg/kg b.w.)
Total protein (g/dl)	6.4 ± 0.3	9.1 ± 0.1 ^a	7.2 ± 0.2 ^d	6.8 ± 0.2 ^d	6.9 ± 0.2 ^d
Albumin (g/dl)	3.6 ± 0.01	7.9 ± 0.04 ^a	4.8 ± 0.1 ^{bd}	3.7 ± 0.4 ^d	3.9 ± 0.3 ^d
SGOT (IU/L)	41.0 ± 1.8	126.6 ± 3.6 ^a	57.2 ± 8.0 ^d	44.3 ± 2.1 ^d	46.2 ± 4.0 ^d
SGPT (IU/L)	37.5 ± 3.7	102.0 ± 4.2 ^a	50.3 ± 6.6 ^d	46.1 ± 3.0 ^d	42.6 ± 2.6 ^d
Acid phosphatase (U/L)	4.6 ± 0.1	10.5 ± 0.4 ^d	6.4 ± 0.7 ^{cd}	5.1 ± 0.4 ^d	4.9 ± 0.2 ^d
Alkaline phosphatase (U/L)	259.9 ± 0.1	745.8 ± 2.1 ^a	394.0 ± 0.9 ^{ad}	263.0 ± 0.6 ^d	265.7 ± 1.2 ^{cd}
Total bilirubin (mg/dl)	1.25 ± 0.02	1.90 ± 0.1 ^a	1.31 ± 0.1 ^d	1.26 ± 0.1 ^d	1.29 ± 0.04 ^d
Direct bilirubin (mg/dl)	0.43 ± 0.01	0.91 ± 0.04 ^a	0.70 ± 0.1 ^{bf}	0.45 ± 0.02 ^d	0.48 ± 0.01 ^d

Values are mean ± standard error mean (SEM) for six animals in each group (n = 6).

^eP < 0.01, designates a significant effect with respect to the group treated with CCl₄ only.

^a P < 0.001, designates a significant effect with respect to control group.

^b P < 0.01, designates a significant effect with respect to control group.

^c P < 0.05, designates a significant effect with respect to control group.

^d P < 0.001, designates a significant effect with respect to the group treated with CCl₄ only.

^f P < 0.05, designates a significant effect with respect to the group treated with CCl₄ only.

group is attributed to membrane fragility in liver tissue and leakage into blood circulation, it is also reported that CCl₄ lowers the capacity of the liver to synthesize albumin (Dubey et al., 1994); however, the retrieval of protein concentration to normalcy confirms its therapeutic potential.

3.3. Serum lipid profile

CCl₄ caused a significant ($P < 0.001$) enhancement in serum LDL, TC and TG levels. Besides, it significantly reduced the serum HDL level, reflecting severe liver damage after CCl₄ intoxication. However, the altered levels of HDL, LDL, TC and TG due to CCl₄ challenge were restored to normal levels by pre-treatment with AqMeOH-Ba (Table 2). The higher dose of AqMeOH-Ba (500 mg/kg b.w.) appears to be highly effective compared to silymarin; however, the treated and silymarin group caused a concomitant increase in HDL levels towards their normal value.

The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from hepatic cells due to altered permeability of membrane (Yadav and Dixit, 2003) is often related with massive liver necrosis (Shyamal et al., 2006). The present study demonstrates that CCl₄ causes large increase in serum marker enzyme activities, and elevation in bilirubin content in serum; evidently the pretreatment of rats with the AqMeOH-Ba markedly restored the changes towards normalcy. The histopathological findings are also in concurrence with the biochemical results. The treatment of AqMeOH-Ba seems to conserve the structural integrity of the hepatocellular membrane. (Fig. 1). The administration of

AqMeOH-Ba was able to maintain the biochemical indicator of liver injury e.g. triglyceride, total cholesterol, HDL and LDL levels to normalcy in the CCl₄ induced animal group, these biochemical restorations may be due to the inhibitory effect of the plant extract on cytochrome P450 (Porchezian and Ansari, 2005).

3.4. Hepatic antioxidant status and histopathological architecture

The hepatic injury induced by CCl₄ caused concomitant hike in LPO and subsequent decline in the levels of enzymatic antioxidants (CAT, GPx, GST, SOD, total reduced glutathione and vitamin C); while, the treatment with AqMeOH-Ba significantly reverted their levels to normalcy in a dose dependent manner. The AqMeOH-Ba at the dose of 500 mg/kg b.w. exhibits an equivalent activity of silymarin (Table 3).

The liver sample of CCl₄ toxicated animals evidenced massive fatty liver with gross necrosis, ballooning degeneration and loss of cellular boundaries. However, administration of the AqMeOH-Ba showed dose dependent reversal of the changes induced by CCl₄ (Fig. 1). The protective nature of the higher dose of the extract was comparable to that of control/silymarin treated groups as evidenced by an appreciable cellular regeneration and reversal of distinct liver characteristic configuration with near normal liver architecture.

The free radical derivatives from CCl₄ are one of the crucial causes of CCl₄ – induced liver injury. The diminution in the activity of SOD, CAT, and GPx may result in an integer of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Das and Vasudevan, 2006). The present study registered enhanced LPO and depressed activities of antioxidant enzymes

Table 2
Effect of aqueous methanolic extract of *B. malabarica* stem bark on serum lipid profile in CCl₄ intoxicated rats.

Parameter	Treatment group				
	Control	CCl ₄ treated	CCl ₄ + extract (250 mg/kg b.w.)	CCl ₄ + extract (500 mg/kg b.w.)	CCl ₄ + silymarin (25 mg/kg b.w.)
HDL (mg/dL)	60.3 ± 0.2	26.8 ± 0.4 ^a	49.9 ± 4.1 ^{bd}	58.9 ± 1.7 ^d	60.0 ± 0.1 ^d
LDL (mg/dL)	79.2 ± 0.01	206.9 ± 0.1 ^a	96.9 ± 7.9 ^d	79.7 ± 6.5 ^d	79.6 ± 0.4 ^d
TC (mg/dL)	158.1 ± 0.2	276.1 ± 0.3 ^a	171.0 ± 5.5 ^{cd}	159.0 ± 1.5 ^d	161.0 ± 2.5 ^d
TG (mg/dL)	93.1 ± 0.1	212.0 ± 2.1 ^a	121.0 ± 9.1 ^{bd}	102.2 ± 0.1 ^d	106.8 ± 4.0 ^d

Values are mean ± standard error mean (SEM) for six animals in each group (n = 6).

^eP < 0.01, designates a significant effect with respect to the group treated with CCl₄ only.

^fP < 0.05, designates a significant effect with respect to the group treated with CCl₄ only.

^a P < 0.001, designates a significant effect with respect to control group.

^b P < 0.01, designates a significant effect with respect to control group.

^c P < 0.05, designates a significant effect with respect to control group.

^d P < 0.001, designates a significant effect with respect to the group treated with CCl₄ only.

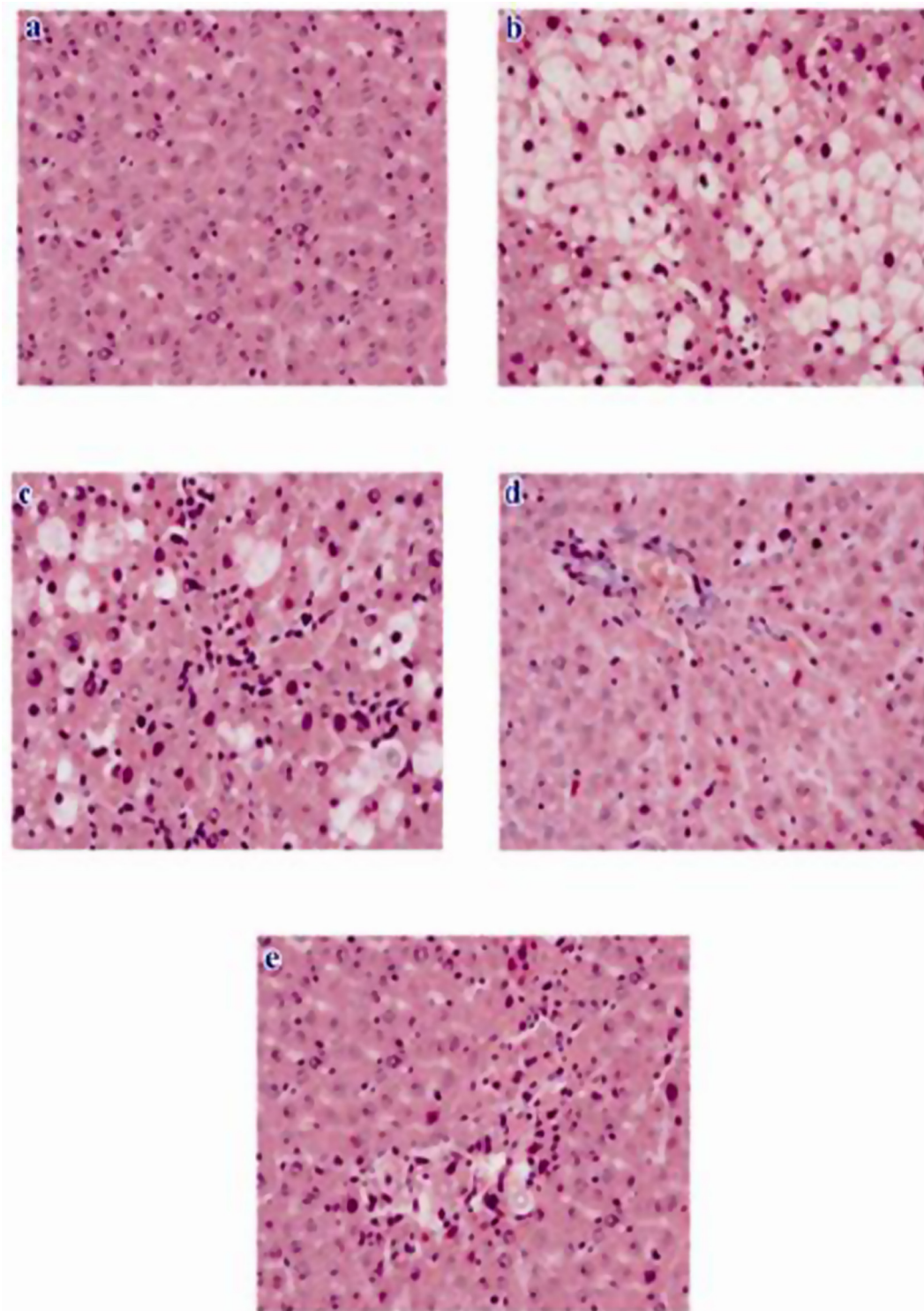


Fig. 1. Photomicrograph showing liver of normal untreated control group (a); CCl₄ induced group (b); CCl₄ + *B. malabarica* stem bark extract (250 mg/kg b.w.) treated group (c); CCl₄ + *B. malabarica* stem bark extract (500 mg/kg b.w.) treated group (d); CCl₄ + silymarin (25 mg/kg b.w.) treated group (e).

(SOD, CAT, GPx and GST) and drastic decreases in the levels of vitamin C and GSH in the experimental rats in response to CCl₄ intoxication. However, AqMeOH-Ba inhibited lipid peroxidation significantly, and recovered the decreased hepatic enzyme levels induced by CCl₄ towards normal levels. The AqMeOH-Ba also enhanced the amount of vitamin C to its normal level. The possible mechanisms underlying the hepatoprotective activity of

AqMeOH-Ba include the prevention of GSH depletion and destruction of free radicals (Das and Vasudevan, 2006). Further, the phytochemical analysis of *B. malabarica* bark has revealed the presence of tannins (Manandhar, 2002), antioxidant flavonols viz., 6,8-di-C-methylkaempferol 3-methyl ether, kaempferol, afzelin, quercetin, isoquercitrin, quercitrin and hyperoside (Kaewamatawong et al., 2008; Farag et al., 2015), and an optically

Table 3
Effect of aqueous methanolic extract of *B. malabarica* stem bark on enzymic and non-enzymic hepatic antioxidant status in CCl₄ intoxicated rats.

Parameter	Treatment group				
	Control	CCl ₄ treated	CCl ₄ + extract (250 mg/kg b.w.)	CCl ₄ + extract (500 mg/kg b.w.)	CCl ₄ + silymarin (25 mg/kg b.w.)
LPO	5.4 ± 1.2	14.7 ± 1.1 ^a	8.5 ± 0.3 ^d	6.4 ± 0.1 ^d	5.7 ± 1.2 ^d
SOD	39.1 ± 0.5	18.8 ± 2.7 ^a	27.9 ± 3.1 ^{df}	33.6 ± 0.8 ^d	35.6 ± 2.3 ^d
CAT	51.9 ± 0.9	30.8 ± 0.8 ^a	48.4 ± 1.4 ^{cd}	50.8 ± 0.1 ^d	49.6 ± 0.2 ^d
GPx	6.0 ± 0.1	1.1 ± 0.2 ^a	5.3 ± 0.1 ^{bd}	5.9 ± 0.1 ^d	5.5 ± 0.1 ^d
GST	6.6 ± 0.4	3.5 ± 0.3 ^a	4.9 ± 0.2 ^{df}	6.2 ± 0.4 ^d	5.7 ± 0.3 ^d
Vitamin C	8.7 ± 0.5	2.0 ± 0.3 ^a	7.3 ± 0.4 ^d	8.0 ± 0.3 ^d	8.4 ± 0.6 ^d
GSH	78.3 ± 5.6	35.3 ± 1.6 ^a	56.4 ± 0.7 ^{cf}	73.8 ± 2.8 ^d	75.0 ± 8.8 ^d

LPO – Lipid peroxidation (μ moles of malondialdehyde (MDA) formed/ mg protein); SOD – Superoxide dismutase (units/mg protein); CAT – Catalase (μ moles of hydrogen peroxide consumed/min/mg protein); GPx – Glutathione peroxidase (μ moles of reduced glutathione utilized/min/mg protein); GST – Glutathione-S-transferase (units/mg protein); Vitamin C – Ascorbic acid (μ moles/mg protein); GSH – Reduced glutathione (μ moles/mg protein).

Values are mean ± standard error mean (SEM) for six animals in each group (n = 6).

^aP < 0.01, designates a significant effect with respect to the group treated with CCl₄ only.

^b P < 0.001, designates a significant effect with respect to control group.

^c P < 0.01, designates a significant effect with respect to control group.

^d P < 0.05, designates a significant effect with respect to control group.

^e P < 0.001, designates a significant effect with respect to the group treated with CCl₄ only.

^f P < 0.05, designates a significant effect with respect to the group treated with CCl₄ only.

active isomer of tartaric acid (Stafford, 1959). The present findings also in concurrence with the previous reports that oxidative damage is substantiated when antioxidants such as ellagitannins, kaempferol, quercetin and rutin administered before or after the induction of oxidative stress (Abo-Salem et al., 2011; Salama et al., 2011; Domitrovic et al., 2012; Panchal and Brown, 2012; Yadav and Mishra, 2015). Moreover, the presence of bioactive compounds viz., phenolic compounds, flavonoids and tannins in *B. malabarica* might be attributed to the antioxidant and hepatoprotective activities, thus exemplifying its potential therapeutic utility.

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Conflict of interest

We declare that we have no conflict of interest.

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