

Antioxidant and Hepatoprotective Effects of *Bauhinia racemosa* against Paracetamol and Carbon Tetrachloride Induced Liver Damage in Rats

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ABSTRACT

The methanol extract of *Bauhinia racemosa* Lam. (Caesalpiniaceae) stem bark was investigated for the antioxidant and hepatoprotective effects in Wistar albino rats. Different groups of animals were administered with paracetamol (500 mg/kg, (p.o.) once in a day for 7 days) and carbon tetrachloride (CCl₄) (30 % CCl₄, 1 ml/kg b.wt. in liquid paraffin 3 doses (i.p.) at 72 h interval). The methanol extract of *Bauhinia racemosa* (MEBR) at the doses of 50, 100 and 200 mg/kg and silymarin 25 mg/kg were administered to the paracetamol and CCl₄ treated rats. The effect of MEBR and silymarin on serum transaminase (SGOT, SGPT), alkaline phosphates (ALP), bilirubin and total protein were measured in the rats induced hepatotoxicity by paracetamol and CCl₄. Further, the effects of the extract on lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were estimated. The MEBR and silymarin produced significant ($P < 0.05$) hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin and lipid peroxidation and significantly ($P < 0.05$) increased the levels of GSH, SOD, CAT and protein in a dose dependant manner. MEBR also showed antioxidant effects on FeCl₂-ascorbate-induced lipid peroxidation in rat liver homogenate and on superoxide scavenging activity. From these results, it was suggested that MEBR could protect the liver cells from paracetamol and CCl₄-induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects of toxic metabolites from paracetamol or CCl₄.

Keywords: *Bauhinia racemosa*, Hepatoprotective effect, Antioxidants, Paracetamol, Carbon tetrachloride

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, through relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. The plant *Bauhinia racemosa* Lam. belongs to the Caesalpiniaceae Family. It is popularly known as Sit-tacha (Tamil) and occurs frequently in India, Ceylon, China and Timor. The stem bark of the plant is an astringent and is used in the treatment of headache, fever, skin diseases, tumors, diseases of the blood, dysentery and diarrhea [1]. β -sitosterol and β -amyrin probably responsible for the related popular use, were isolated from the stem bark of this plant [2]. Beside these compounds, at least five flavonols (Kaempferol and Quercetin) and two coumarin (scopoletin and scopolin) were also isolated from the leaf [3]. Stilbene (Resveratrol) was isolated from the heartwood of *B. racemosa*

[4]. Pharmacological studies of the plant revealed that the ethanol extract of leaves of *B. racemosa* shows analgesic, antipyretic, anti-inflammatory and antispasmodic [5], and antimicrobial activity [6]. The fresh flower buds of the plant showed antiulcer activity [7]. The cytotoxicity against CA-9 KB in cell culture, hypotensive and hypothermic activities were reported from the hydroalcoholic extract of *B. racemosa* [8].

Paracetamol (Acetaminophen) is a widely used antipyretic and analgesic, produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates [9, 10]. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 [11], to a highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI) [12]. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercap-

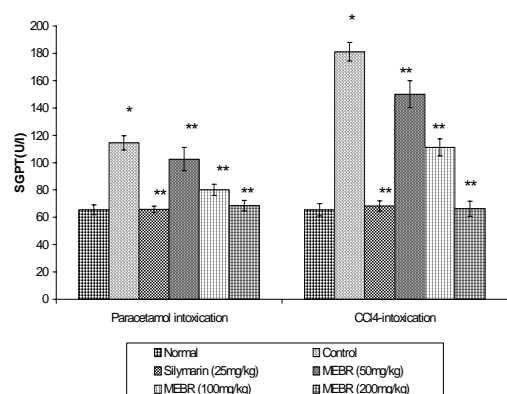


Fig 1. Effect of MEBR on serum GPT levels of Paracetamol and CCl₄-intoxicated rats. Results are presented as the mean \pm S.E.M. (n=6).

* P < 0.001 control group compared with the normal group.
** P < 0.05 MEBR treated groups compared with the control.

aluric acid [13]. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of protein and alters the homeostasis of calcium after depleting GSH.

The experimental intoxication induced by carbon tetrachloride (CCl₄) is widely used for modeling liver injury in rats. Hepatotoxicity is connected with severe impairment of cell protection mechanisms. The location of liver injury is defined mainly by the biotransformation of CCl₄, which is cytochrome P-450 dependant. Free radicals initiate the process of lipid peroxidation, which is generally cause of inhibition of enzyme activity [14, 15]. It is now generally accepted that the hepatotoxicity of CCl₄ is the result of reductive dehalogenation, which is catalyzed by P-450, and which forms highly reactive trichloromethyl free radical. This readily interacts with molecular oxygen to form the trichloromethyl peroxy radical. Both trichloromethyl and its peroxy radical are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage and by doing so playing a significant role in pathogenesis of diseases [16].

Plant derived natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [17-19]. There has been growing interest in the analysis of certain flavonoids, triterpenoids and steroids stimulated by intense research into their potential benefits to human health. One of their main properties in this regard is their antioxidant activity, which enables them to attenuate the development of tumor and inflammatory disease. Antioxidant plays an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases. Realizing the fact, this research was carried out to evaluate the antioxidant and hepatoprotective activity of methanol extract of *Bauhinia racemosa*

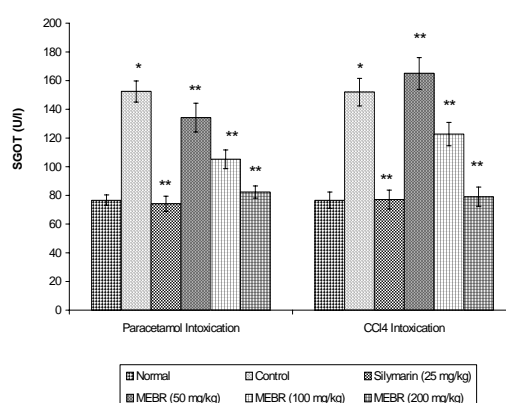


Fig 2. Effect of MEBR on serum GOT levels of Paracetamol and CCl₄-intoxicated rats. Results are presented as the mean \pm S.E.M. (n=6).

* P < 0.001 control group compared with the normal group.
** P < 0.05 MEBR treated groups compared with the control.

(MEBR) against paracetamol and CCl₄-induced liver damage in rats.

MATERIALS AND METHODS

Plant Material

The plant *Bauhinia racemosa* Lam. (Family: Caesalpiniaceae) a small crooked, bushy tree with drooping branches, found throughout India ascending to an altitude of 1,650 m from sea level. The stem bark was collected in the month of March 2003 from the Kolli Hills, Tamil Nadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Shibpur, Kolkata, India, and the Voucher specimen (No. GMS-1) was retained in our laboratory for future reference. The dried powder material of the stem bark of *Bauhinia racemosa* was extracted with methanol (Yield 9.25%) in a soxhlet apparatus. The methanol extract was then distilled, evaporated and dried in vacuum. The chemical constituents of the extract were identified by qualitative analysis followed by their confirmation by thin layer chromatography, which indicate the presence of flavonoids, triterpenoids, steroids and tannins.

Animals

Studies were carried out using Wistar albino rats (150–180 g) of either sex were used. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in polyacrylic cages (38 \times 23 \times 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 \pm 2°C) with dark and light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

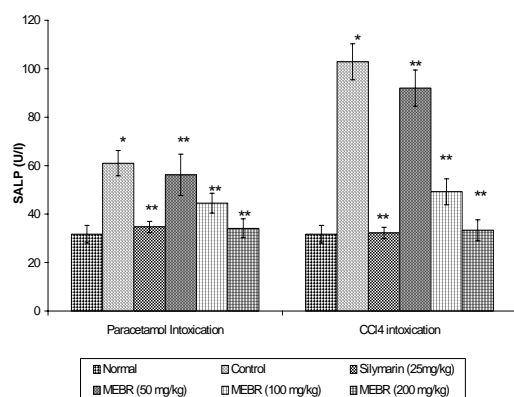


Fig 3. Effect of MEBR on serum ALP levels of Paracetamol and CCl_4 -intoxicated rats. Results are presented as the mean \pm S.E.M. (n=6).

* $P < 0.001$ control group compared with the normal group.
** $P < 0.05$ MEBR treated groups compared with the control.

Drugs and Chemicals

Silymarin was purchased from Micro labs Tamilnadu India, 1-Chloro-2, 4-dinitrobenzene [CDNB], Bovine serum albumin (Sigma chemical St. Louis, MO, USA), Thiobarbituric acid, Nitroblue tetrazolium chloride (NBT) (Loba Chemie, Bombay, India), 5,5'-dithio bis-2-nitrobenzoic acid (DTNB), Carbon tetrachloride, (SICCO Research Laboratory, Bombay). The solvents and/or reagents obtained were used as received.

Toxicity study

For toxicity studies, groups of 10 mice were administered (i.p.) with test compounds in the range of doses 100-1750 mg/kg. And the mortality rates were observed after 72 h. The LD_{50} was determined using the graphical methods of Litchfield and Wilcoxon [20].

Paracetamol-induced liver damage in rats

Healthy albino rats were divided into 6 groups of 6 animals in each. Group 1, which served as normal, received normal saline (5 ml/kg b.wt.). Group 2 received paracetamol (500 mg/kg p.o) once daily for 7 days (control). Group 3, 4, and 5 received paracetamol (500 mg/kg, p.o.) and MEBR (50, 100 and 200 mg/kg p.o.) simultaneously for 7 days. Group 6 received paracetamol (500 mg/kg, p.o.) and standard drug silymarin (25 mg/kg p.o) simultaneously for 7 days [21]. The biochemical parameters were determined after 18 h fasting of the last dose.

Carbon tetrachloride-induced liver damage in rats

Healthy albino rats were divided into 5 groups each containing 6 animals. Group 1 (Control) received 30% CCl_4 in liquid paraffin (1 ml/kg body weight, i.p.). Group 2, 3 and 4 received MEBR 50, 100 and 200 mg/kg p.o. respectively and Group 5 received standard drug Silymarin (25 mg/kg p.o) once in a day and CCl_4 as mentioned above. Treatment duration was 10 days

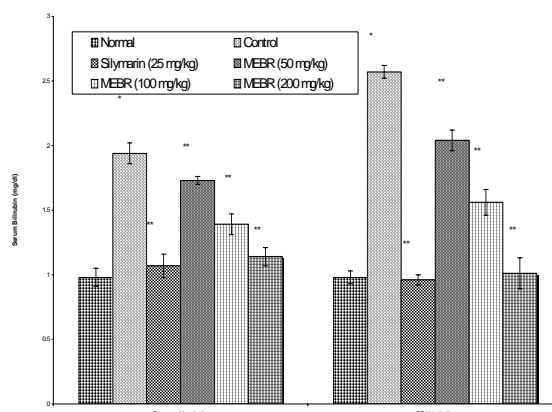


Fig 4. Effect of MEBR on serum bilirubin levels of Paracetamol and CCl_4 -intoxicated rats. Results are presented as the mean \pm S.E.M. (n=6).

* $P < 0.001$ control group compared with the normal group.
** $P < 0.05$ MEBR treated groups compared with the control.

and the dose of CCl_4 was administered after every 72 h [22]. Animals were sacrificed 24 h after the last injection. Blood was collected, allowed to clot and serum separated. The liver was dissected out and used for biochemical studies.

Biochemical studies

The blood was obtained from all animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters namely SGPT, SGOT Bergmeyer et al. (1978) [23], SALP King, 1965 [24], serum bilirubin by the method of Malloy and Evelyn, (1937) [25], and protein content was measured by the method of Lowry et al. (1951) [26].

After collection of blood samples the rats were sacrificed and their livers excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation by the method of Ohkawa et al. (1979) [27]. A part of homogenate after precipitating proteins with Trichloric acetic acid (TCA) was used for estimation of glutathione by the method of Ellman (1959) [28]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C . The supernatant thus obtained was used for the estimation of SOD by the method described by Kakkar et al. (1984) [29] and CAT activities was measured by the method of Aebi (1974) [30].

Lipid peroxidation

The tissues were then homogenized in 0.1 M buffer (pH 7.4) with a Teflon-glass homogenizer. Lipid peroxidation in this homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily, according to the method of Ohkawa et al.

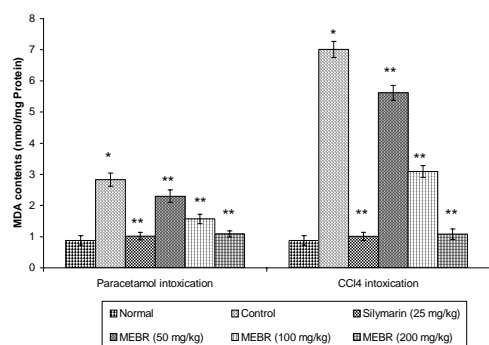


Fig 5. Effect of MEBR on serum protein levels of Paracetamol and CCl₄-intoxicated rats. Results are presented as the mean \pm S.E.M. (n=6).

* P < 0.001 control group compared with the normal group.

** P < 0.05 MEBR treated groups compared with the control.

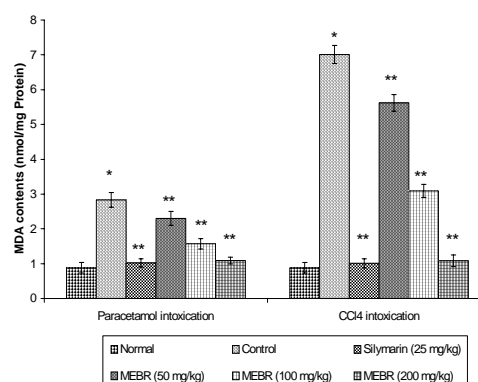


Fig 6. Anti-lipid Peroxidative effect of MEBR in the Liver of Paracetamol and CCl₄-intoxicated rats. Results are presented as the mean \pm S.E.M. (n=6).

* P < 0.001 control group compared with the normal group.

** P < 0.05 MEBR treated groups compared with the control.

(1979). 0.2 ml of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 8% TBA were added. The volume of the mixture was made up to 4 ml with distilled water and then heated at 95°C on a water bath for 60 min using glass balls as condenser. After incubation the tubes were cooled to room temperature and final volume was made to 5 ml in each tube. 5.0 ml of butanol:pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its OD read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Estimation of GSH

To measure the reduced glutathione (GSH) level, the tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was taken. The procedure was followed initially as described by Ellman 1959. The homogenate was added with equal volume of 20% trichloroacetic acid (TBA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200 μ l) was then transferred to a new set of test tubes and added 1.8 ml of the Ellman's reagent (5, 5'-dithio bis-2-nitrobenzoic acid) (0.1mM) was prepared in 0.3M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes make upto the volume of 2ml. After completion of the total reaction, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH.

Estimation of SOD

SOD activity of the liver tissue was analyzed by the method described by Kakkar et al. (1984). Assay mixture contained 0.1 ml of sample, 1.2 ml of sodium pyro-

phosphate buffer (pH 8.3, 0.052 M), 0.1 ml phenazine methosulphate (186 μ M), 0.3 ml of 300 μ M nitroblue tetrazolium, 0.2 ml NADH (750 μ M). Reaction was started by addition of NADH. After incubation at 30°C for 90 s, the reaction was stopped by the addition of 0.1 ml glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 ml of n-butanol. Mixture was allowed to stand for 10 min, centrifuged and butanol layer was separated. Color intensity of the chromogen in the butanol layer was measured at 560 nm spectrophotometrically and concentration of SOD was expressed as units/mg protein.

Estimation of CAT

Catalase activity was measured by the method of Aebi (1974). 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H₂O₂. The rate of decomposition of H₂O₂ was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein.

FeCl₂-ascorbic acid stimulated lipid peroxidation in liver homogenate

The Wister albino rats weighing 175-200 g were killed by decapitation and their liver tissues were quickly removed. A 2 g portion of liver tissue was sliced and then homogenized with 10 ml of 150 mM KCl Tris-HCl buffer (pH 7.2). The reaction mixture was composed of 0.25 ml of liver homogenate, 0.1 ml of Tris-HCl buffer (pH 7.2), 0.05 ml of 0.1 mM ascorbic acid, 0.05 ml of 4 mM FeCl₂ and 0.05 ml of various concentrations of MEBR. The products of lipid peroxidation were quantified by the formation of the thiobarbituric acid-reactive material, MDA [31]. 1,1,3,3-Tetraethoxypropane was used as a standard for calibration of MDA. Appropriate controls were used to eliminate any possible interference with the thiobarbituric acid assay.

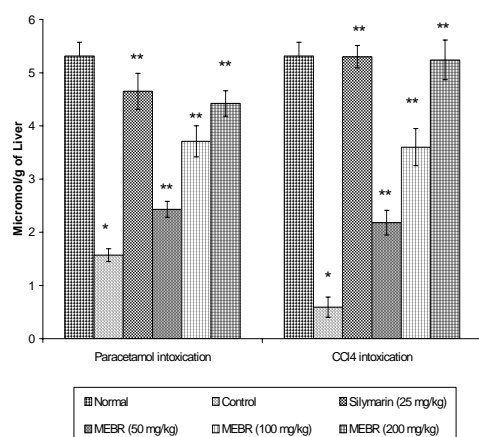


Fig 7. Effect of MEBR on Glutathione content of Paracetamol and CCl₄-intoxicated rat Liver. Results are presented as the mean \pm S.E.M. (n=6).

* P < 0.001 control group compared with the normal group.

** P < 0.05 MEBR treated groups compared with the control.

Assay of Superoxide scavenging activity

Superoxide was generated using Xanthine (100 μ M) and xanthine oxidase (0.02 U) with or without various concentrations of added MEBR, in 1ml of 10 mM KH₂PO₄-KOH buffer, pH 7.4, detected using nitroblue tetrazolium (100 μ M) and quantified spectrophotometrically at 550 nm [32]. Superoxide dismutase (100 U/ml) was used as a reference inhibitor.

Statistical analysis

All experiments were repeated at least three times. Results are reported as means \pm S.E.M. ANOVA was used to evaluate differences between groups. If significance was observed between groups, the Student's t-test was used to compare the means of specific groups, with P < 0.05 considered as significant.

RESULTS

Acute toxicity

Results of treatment of mice with MEBR have been summarized in Fig 11, LD₅₀ value of MEBR was found to be log 2.98 and its antilog is 955. Therefore, intraperitoneally the LD₅₀ value of MEBR in mice is 955 mg/kg body weight.

Effect of MECEB on serum enzymes, bilirubin and protein

Changes in the activities of serum enzymes (GPT, GOT and ALP), bilirubin and total protein content in the serum of paracetamol and CCl₄-induced liver damage in rats as evidence from Fig 1-5. The level of serum marker enzymes GPT, GOT, ALP and bilirubin were found to be significantly increased and protein content significantly decreased in paracetamol and CCl₄-induced liver damage rats when compared with the

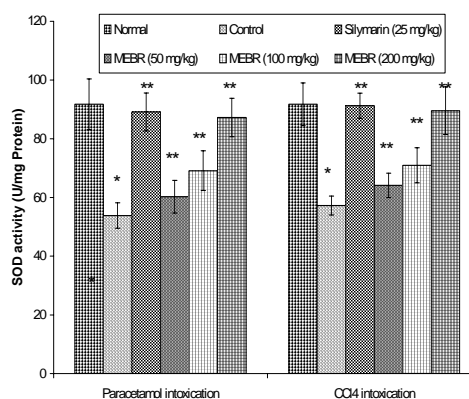


Fig 8. SOD activity following treatment with MEBR in the Liver of Paracetamol and CCl₄-intoxicated rats Results are presented as the mean \pm S.E.M. (n=6).

* P < 0.001 control group compared with the normal group.

** P < 0.05 MEBR treated groups compared with the control.

normal group (P<0.001). Whereas treatment with MEBR at the dose of 50, 100 and 200 mg/kg showed decreased the activity of serum transaminase, ALP, bilirubin and increased the protein content in paracetamol and CCl₄-induced liver damage in rats compared to that of control groups (P<0.05). Silymarin (25 mg/kg) also significantly decreased the levels of serum enzymes, bilirubin and increased the protein content in paracetamol and CCl₄-treated groups as compared with the respective control group.

In vivo Lipid peroxidation

The localization of radical formation resulting in lipid peroxidation, measured as MDA in rat liver homogenate is shown in Fig 6. Malondialdehyde (MDA) contents in the liver homogenate were increased in paracetamol control group (2.83-nmol/mg protein) compared to normal group (0.88 nmol/mg protein, p < 0.001). MDA level of MEBR 50, 100 and 200 mg/kg groups (2.3, 1.57 and 1.09 nmol/mg protein, P < 0.05) were inhibited by 27.2, 36.7 and 89.2 % compared to paracetamol control group. MDA content in the liver homogenate was increased in CCl₄ control group (7.01 nmol/mg protein, P < 0.001) compared to normal group. MDA level of MEBR 50, 100 and 200 mg/kg group (5.62, 3.90 and 1.08 nmol/mg protein, P < 0.05) were inhibited by 22.7, 50.7 and 81.2 % compared to CCl₄ control. At the same time, the effect of silymarin 25 mg/kg on MDA levels in paracetamol and CCl₄ were inhibited by 92.8 and 98.3% respectively.

GSH level in liver tissues

The effect of MEBR on glutathione content in the liver is shown in Fig 7. GSH level of liver homogenate in paracetamol control group (1.57 μ mol/g of liver) was found to lower than in normal group (5.31 μ mol/g of liver, P < 0.001). GSH level of MEBR 50, 100 and 200

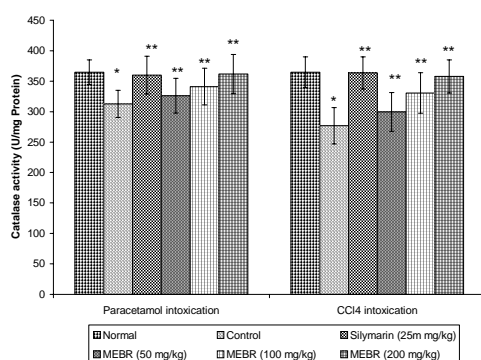


Fig 9. Catalase activity following treatment with MEBR in the Liver of Paracetamol and CCl₄-intoxicated rats. Results are presented as the mean \pm S.E.M. (n=6).

* P < 0.001 control group compared with the normal group.

** P < 0.05 MEBR treated groups compared with the control.

mg/kg group in liver total homogenate was pronounced with 2.43, 3.71 and 4.42 μ mol/g of liver. These results were increased by 22.9, 57.2 and 76.2% as compared to paracetamol control group. GSH level in normal group (5.31 μ mol/g of liver) was measured to be higher than in CCl₄ control group (0.59 μ g/mg protein). GSH level of MEBR 50, 100 and 200 mg/kg groups (2.18, 3.60 and 5.24 μ mol/g of liver, P < 0.05) were increased by 33.7, 63.8, and 97.2% respectively as compared to CCl₄ control group. Silymarin almost completely restored the glutathione level in paracetamol and CCl₄ treated groups to the normal level.

SOD activity in liver tissues

The effect of MEBR on SOD activity in liver is shown in Fig 8. SOD activity of the liver total homogenate in paracetamol control group (53.83 U/mg protein) was found to be lower than in normal group (91.76 U/mg protein, P < 0.001). SOD activities of MEBR 50, 100 and 200 mg/kg groups (60.25, 69.15 and 87.25 U/mg protein) were increased by 17.6, 40.0 and 88.1 % respectively. SOD activity in CCl₄ control group (57.23 U/mg protein) was examined to be lower than in normal group (91.76 U/mg protein, P < 0.001). SOD activities in MEBR 50, 100 and 200 mg/kg groups (64.14, 70.93 and 89.56 U/mg protein, P < 0.05) were observed to be higher than in CCl₄ control group. SOD activities of MEBR 50, 100 and 200 mg/kg were improved by 20.9, 39.7 and 87.7 % respectively. Silymarin 25 mg/kg also restored the SOD activity in both paracetamol and CCl₄ treated groups.

Catalase activity in liver tissues

CAT activities in the liver homogenate were shown in Fig 9. CAT activity of liver total homogenate in paracetamol control group (312.65 U/mg protein) was found to be conspicuously lower than in normal group

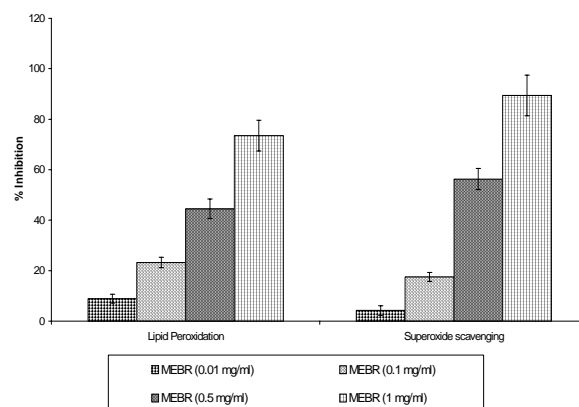


Fig 10. Inhibitory effects of methanol extract of *B. racemosa* (MEBR) on FeCl₂-Ascorbic acid stimulated Lipid peroxidation and Superoxide scavenging activity. Values are presented as mean of the percentage inhibition \pm S.E.M. for three independent experiments, performed in triplicate. Rat liver homogenates were stimulated with FeCl₂-Ascorbic acid in the presence or absence of MEBR and lipid peroxidation was measured. Superoxide was generated by the oxidation of Xanthine/Xanthine oxidase in the presence of MEBR and scavenging activity was measured

(364.65 U/mg protein). Total homogenated liver CAT activities in MEBR 50, 100 and 200 mg/kg (326.15, 341.15 and 351.73 U/mg protein, P < 0.05) groups were increased by 25.9, 54.8 and 75.2 % respectively. In addition, CAT activity of CCl₄ control group (276.87 U/mg protein) was measured to be strikingly lower than in normal group (364.65 U/mg protein, P < 0.001). Liver CAT activities in MEBR 50, 100 and 200 mg/kg (299.6, 330.75 and 357.63 U/mg protein, P < 0.05) groups were increased by 25.9, 61.4 and 82.9 % respectively. MEBR and silymarin completely restored the enzyme activity to the normal level at the respective doses of 200 mg/kg and 25 mg/kg.

In vitro lipid peroxidation and free radical scavenging activity

Effect of MEBR on FeCl₂-ascorbic acid stimulated lipid peroxidation and superoxide-scavenging activity is summarized in Fig 10. In order to determine the antioxidant effect of MEBR in terms of the mechanisms of its hepatoprotective effect, anti-lipid peroxidation in liver homogenate and superoxide scavenging activity of MEBR were investigated. Consistent with the result of carbon tetrachloride-induced hepatic lipid peroxidation, MEBR showed a dose-dependant inhibition of the FeCl₂-ascorbic acid stimulated lipid peroxidation with an IC₅₀ value of 0.71 mg/ml in liver homogenate. MEBR also showed superoxide-scavenging activity with an IC₅₀ value of 0.53 mg/ml.

DISCUSSION

In the assessment of liver damage by paracetamol and CCl₄ hepatotoxin, the determination of enzyme levels such as SGPT and SGOT is largely used. Necrosis or membrane damage releases the enzyme into circulation; therefore, it can be measured in serum. High levels of

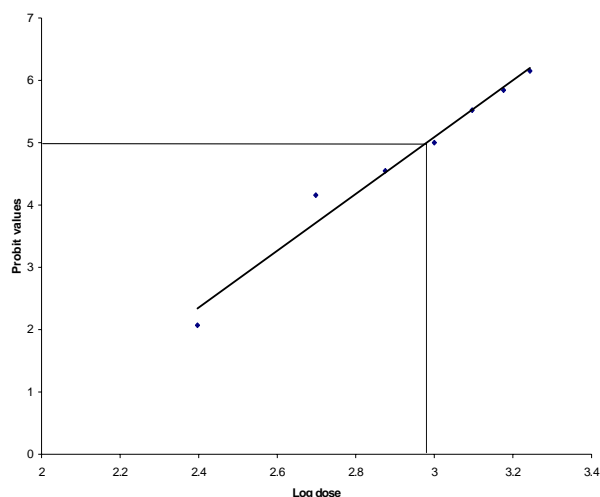


Fig 11. LD₅₀ determination of methanolic extract of *Bauhinia racemosa* (MEBR).

SGOT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury [33]. Our results using the model of paracetamol and CCl₄-induced hepatotoxicity in the rats demonstrated that MEBR at the different doses caused significant inhibition of SGPT and SGOT levels. Serum ALP and bilirubin levels on the other hand, are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [34]. Our results using the model of paracetamol and CCl₄-induced hepatotoxicity in rats demonstrated that MEBR at different doses caused significant inhibition of SALP and bilirubin levels. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell.

In recent years, attention has been focused on the role of biotransformation of chemicals to highly reactive metabolites that initiate cellular toxicity. Many compounds, including clinically useful drugs, can cause cellular damage through metabolic activation of the chemical to highly reactive compounds such as free radicals, carbenes and nitrenes. CCl₄ has probably been studied more extensively both biochemically and pathologically than any other hepatotoxin. CCl₄ hepatotoxicity depends on the reductive dehalogenation of CCl₄ catalysed by Cyt 450 in the liver cell endoplasmic reticulum leading to the generation of an unstable complex CCl₃ radical. This trichloromethyl radical reacts rapidly, which is reported as a highly reactive species. These free radicals attack microsomal lipids leading to its peroxidation and also covalently binds to microsomal lipids and proteins ultimately initiating a site of secondary biochemical processes which is the ultimate cause for the unfolding of the panorama of pathological consequences of CCl₄ metabolism [35]

Cells have a number of mechanisms to protect themselves from the toxic effects of ROS. SOD removes superoxide (O₂) by converting it to H₂O₂, which can be rapidly converted to water by CAT and glutathione peroxidase (GPx) [36]. In addition, a large reserve of reduced glutathione is present in hepatocytes and red blood cells for detoxification of xenobiotics or free radicals. However, oxidative stress results in toxicity when the rate at which the ROS are generated exceeds the cell capacity for their removal. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. MDA is one of the end products in the lipid peroxidation process [37]. In order to elucidate the protection mechanism of MEBR, paracetamol and CCl₄ induced rat liver, after MEBR injection, was examined at lipid peroxide levels and antioxidative enzyme activities.

Liver cell injury induced by CCl₄ involves initially the metabolism of CCl₄ to trichloromethyl free radical by the mixed-function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanisms link CCl₄ metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from CCl₄ metabolism or from peroxidative degeneration of membrane lipids [38]. In our study, elevations in the levels of end products of lipid peroxidation in liver of rat treated with paracetamol and CCl₄ were observed. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with MEBR significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of MEBR is due to its antioxidant effect.

GSH is widely distributed in cells. GSH is an intracellular reductant and plays major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects, for example, a decrease in the rate of gluconeogenesis or an increase in glycogenolysis. The concept of a glutathione-SH threshold for drug detoxification was discussed by Jollow [40]. GSH is a naturally occurring substance that is abundant in many living creatures. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury. For example, liver injury included by consuming alcohol or by taking drugs like acetaminophen, lung injury by smoking and muscle injury by intense physical activity [41], all are known to be correlated with low tissue levels of GSH. From this point of view, exogenous MEBR supplementation might provide a mean of recover reduced GSH levels and to prevent tissue disorders and injuries. The present study, we have demonstrated the effectiveness of MEBR by using paracetamol and CCl₄ induced rats, which are known models for both hepatic GSH depletion and injury.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes such as SOD, CAT and GPx system. [39]. The SOD dismutates superoxide radicals O_2^- into H_2O_2 plus O_2 , thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. In this study, SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. The observed increase of SOD activity suggests that the MEBR have an efficient protective mechanism in response to ROS. And also, these findings indicate that MEBR may be associated with decreased oxidative stress and free radical-mediated tissue damage.

CAT is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of MEBR increases the activities of catalase in paracetamol and CCl_4 -induced liver damage rats to prevent the accumulation of excessive free radicals and protects the liver from paracetamol and CCl_4 intoxication.

In vitro lipid peroxidation in a liver homogenate can proceed in a non-enzymatic way. The process is induced by ascorbate in the presence of Fe^{2+}/Fe^{3+} , and it has been reported that Fe^{2+} and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and mitochondria. In order to clarify the mode of action of MEBR, in vitro lipid peroxidation experiments were carried out. According to the result obtained, MEBR inhibited $FeCl_2$ -ascorbic acid-stimulated lipid peroxidation in liver homogenate (Fig 10). Moreover, our experimental results demonstrate that MEBR exercises free radical scavenging activity on the superoxide radicals generated using xanthine-xanthine oxidase system (Fig 10), and may therefore, act by scavenging free radicals and reactive oxygen species formed during the paracetamol and carbon tetrachloride metabolism.

It has been reported that *Bauhinia racemosa* contains flavonoids, triterpenoids and steroids [2, 3]. A number of scientific reports indicated certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties [17-19]. Presence of those compounds in MEBR may be responsible for the protective effect on paracetamol and CCl_4 -induced liver damage in rats.

In conclusion, the results of this study demonstrate that MEBR has a potent hepatoprotective action upon paracetamol and carbon tetrachloride-induced hepatic damage in rats. Our results show that the hepatoprotective effects of MEBR may be due to its antioxidant and free radical scavenging properties. Further, investigation is underway to determine the exact phytoconstituents that is responsible for its hepatoprotective effect.

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