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 Sittacha (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 (Resvertrol) 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 antivirusmodic [5], and antimicrobial activity [6]. The fresh flower buds of the plant showed antifulcer activity [7]. The cytotoxicity against CA-9 KB in cell culture, hypertensive and hypotermic 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 excretory 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-
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**Fig 1.** Effect of MEBR on serum GPT levels of Paracetamol and CCl4-intoxicated rats. Results are presented as the mean ± 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 2.** Effect of MEBR on serum GOT levels of Paracetamol and CCl4-intoxicated rats. Results are presented as the mean ± 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: Caesalpinaceae) 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 soxlet 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 × 23 × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 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 conditions for 10 days before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Paracetamol intoxication CCl4-intoxication

![Graph](image1.png)

![Graph](image2.png)

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Fig 2. Effect of MEBR on serum GOT levels of Paracetamol and CCl4-intoxicated rats. Results are presented as the mean ± 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 LD50 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% CCl4 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 CCl4 as mentioned above. Treatment duration was 10 days and the dose of CCl4 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.
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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 µl) 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 up 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 pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml phenazine methosulphate (186 µM), 0.3 ml of 300 µM nitroblue tetrazolium, 0.2 ml NADH (750 µM). 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 FeCl3 and 0.05 ml of various concentrations of MEBR. The products of lipid peroxidation were quantified by the formation of the thioarbituric acid-reactive material, MDA [31]. 1,1,3,3-Tetraethoxyxyp propane was used as a standard for calibration of MDA. Appropriate controls were used to eliminate any possible interference with the thioarbituric acid assay.
Fig 7. Effect of MEBR on Glutathione content of Paracetamol and CCl4-intoxicated rat Liver. Results are presented as the mean ± 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 µM) 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 µM) 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 ± 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 MECB 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 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 µmol/g of liver) was found to lower than in normal group (5.31 µmol/g of liver, P < 0.001). GSH level of MEBR 50, 100 and 200
Control

MEBR (0.1 mg/ml)
Silymarin (25 mg/kg)
MEBR (200 mg/kg)
MEBR (1 mg/ml)
MEBR (100 mg/kg)

respectively. SOD activity in CCl4 control group (57.23 U/mg protein) were increased by 17.6, 40.0 and 88.1 % 100 and 200 mg/kg groups (60.25, 69.15 and 87.25 U/mg protein, P < 0.001). SOD activities of MEBR 50, 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 (299.6, 330.75 and 357.63 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). Total homogenized 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). 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, 51.4 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.

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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 (53.83 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.

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

**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 ± 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.
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 peroxide (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 peroxi-degeneration of membrane lips [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 hepato-protection 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 induced 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.

SGPT 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 CCl•- 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].
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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. [29]. The SOD dismutates superoxide radicals O$_2^-$ into H$_2$O$_2$ 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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