ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

IN VIVO ANTIOXIDANT ACTIVITY OF *BOMBAX CEIBA BARK AND LEAVES* AGAINST CARBON TETRACHLORIDE INDUCED LIVER TOXICITY IN RATS

Sarita Karole[1, 2*], Girendra Kumar Gautam[1, 3], Shailesh Gupta[4]

[1]*Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan, India*
[2]*Oriental College of Pharmacy, Bhopal, M.P., India*
[3] *Shri Ram College of Pharmacy, Muzaffarnagar, U.P., India*
[4]*SRK, University, Bhopal, M.P., India*
**Corresponding author: simrankare@gmail.com*

ABSTRACT

Antioxidants are imperative substances which possess the facility to protect the body from injure caused by free radical induced oxidative stress. A diversity of free radical scavenging antioxidants exist within the body, many of them are derived from dietary sources like fruits, vegetables and teas. In this study antioxidant activity of ethanolic extract of *Bombax ceiba* bark and leave was investigated using CCl_4 intoxicated rat liver as the experimental model. Rats divided into five groups were administered with CCl_4 and CCl_4 along with ethanolic extract of bark and leave of *Bombax ceiba* (200 mg/kg b.wt) for 9 days. In the last day of activity, rats were anaesthetized and blood samples were collected for serum separation. Biochemical analysis such as lactate dehydrogenase (LDH) was done in serum. Liver tissue was used for glutathione (GSH) level, gluatathione reductase (GRD), glutathione-S-transferase (GST)and catalase (CAT) analysis and histopathology studies. The activities of their tissue antioxidant enzymes increased significantly in animals consequent to CCl_4 (Group II)-induced hepatic damage as compared to the normal animals (Group I). Oral administration of leaves and bark extract at the dose of 200mg/kg (Group IV/V) showed significant decreased in LDH, CAT, GRD, GST and GSH when compared with CCl4-treated rats. Treatment with *bombax ceiba* bark extract (Group V) showed only marginal decreased in the level of antioxidant enzymes in liver homogenate compared with CCl4-treated rats, but leave extract give acceptable activity. Thus, the activities of LDH, CAT, GRD, GST and GSH were restored by *bombax ceiba* treatment. Histopathological examination showed lowered liver damage in *Bombax ceiba* leave-treated groups. The findings show that *Bombax ceiba* offers better protection against the free radical toxicity of CCl_4.

Keywords: *Bombax ceiba,* CCl_4, Free radical, Antioxidant, Liver

1. INTRODUCTION

Liver plays a vital role in metabolism, detoxification and excretion of many xenobiotic compounds. Because of its anatomical position and its great ability for xenobiotic metabolism, it is regularly a target for toxic chemicals. Although viral infection is one of the major causes of liver injury, xenobiotics, extreme drug therapy, environmental contaminants and chronic alcohol drinking can also cause hepatic injury. Cancer chemotherapeutic drugs caused liver toxicity has been widely reported [1]. Most of these toxic chemicals have been reported to create free radicals and reactive oxygen species which are the major offenders in liver pathogenesis [2]. The indecent balance between reactive metabolites production and antioxidant defense results in oxidative stress which controls the cellular functions leading to various pathological conditions. Free radical-mediated lipid per oxidation induced by these chemicals play a vital role in various steps that initiate and regulate the succession of liver diseases separately of the agent in its origin [3, 4]. The treatment for liver diseases has become an exigent problem of the modern medicine. A number of herbal preparations have been promoted for treating liver diseases. Research explorations conducted on several plant products as liver protective are well documented [5, 6]. Because free radicals and ROS play a central role in liver diseases pathology and development, dietary antioxidants have been projected as therapeutic agents to counteract liver damage [7-9]. Additionally, recent studies have recommended that natural antioxidants in complex mixtures ingested with the diet are more efficient than pure compounds in preventing

oxidative stress-related pathologies due to particular interactions and synergism [4]. It is evident that there is an escalating demand to evaluate the antioxidant properties of direct plant extract [10]. And there is a necessitate for screening more bioactive plant products with antioxidant properties. Carbon tetrachloride (CCl_4) is a typical hepatotoxin broadly used in various experimental models. CCl_4 induces liver injuries by mediating through the formation of its reactive intermediates such as trichloromethyl radical (CCl_3•) and its derivative trichloromethyl peroxy radical (CCl_3 OO•), produced by cytochrome P450 of liver microsomes. These free radicals generated react with membrane lipids leading to their peroxidation [11]. *Bombax ceiba* (syn. Bombax malabaricum) is an imperative medicinal plant of tropical and subtropical India commonly known as Silk Cotton Tree or Semal [12]. It is a tall deciduous tree, with straight butteressed trunk and wide spreading branches. Almost every part of this plant is used as medicine for curing maximum number of ailments. Its bark is mucilaginous, demulcent and emetic and is used in healing wounds; bark paste is good for skin eruptions [13, 14]. Cotton tree has been used expansively for treatment of some diseases like inflammation, algesia, hepatotoxicity and hypertension, as well as for antiangiogenic and antioxidant activities [15-18]. Bark contains lupeol, saponins, tannins, gums and 4, 5, 7-trihydroxy-flavone-3-O-β-Dglucopyranosyl (1-4)-α-L-rhamnopyranoside [19]. In other studies secondary metabolites like triterpenoids obtained from plants influenced both cellular and humoral immune responses in rats and mice and it has been reported that polyphenols, flavone are effective scavengers of free radicals, and also helps in the modulation of immune functions [20]. Few studies on *Bombax ceiba* proved to be effective in protecting liver from CCl_4 toxicity. However, antioxidant mediated protective effect of this herb on CCl_4 induced hepatopathogenesis have not been reported earlier. The present study primarily focuses on the antioxidant defense mechanisms of the plant extracts.

2. MATERIAL & METHODS

2.1. Material

The plant part (leaves and bark) of *bombax ceiba* was collected from local area of Bhopal (M.P.) in the month of February 2017. All the other chemicals and reagents used in this study were of AR grade and were purchased from SD Fine Chemicals, Mumbai and Hi-media Laboratories Pvt. Ltd, Mumbai.

2.2. Methods

2.2.1. Extraction

Dried pulverized leaves and bark of *Bombax ceiba* were placed in thimble of soxhlet apparatus. Soxhlation was performed at 60-80°C using petroleum ether as non-polar solvent at first. Exhausted plant material (marc) was dried and then extracted with ethanol separately. Soxhlation was continued till no colour was observed in siphon tube. For confirmation of exhausted plant marc (i.e. completion of extraction), colorless solvent was collected from siphon tube and completion of extraction was confirmed by absence of any residual solvent, The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [21].

2.2.2. Animals

Male wistar rats weighing about 200±20 gm were used for the study. They were housed in well conditioned room with 12 h light/12 h dark photoperiod. They were fed with standard animal feed (Lipton India, Bangalore, India) and water *ad libitum*. Experiments were conducted in accordance with the institutional ethical committee guidelines.

2.2.3. Experimental design

Rats were divided into five groups containing five rats each. Group 1 served as control, Group 2 was administered carbon-tetrachloride (negative control), Group 3 was administered with Silymarin (10 mg/kg) and Group 4, 5 were administered sample extract for 9 days. The rats of the group 1& 2 were simultaneously administered saline until the 9th day. On the 9th day the rats of the group 3-5 were given a single dose of carbon-tetrachloride (1:1) in olive oil at 1ml/kg of body weight 6 hrs after the last dose of extract/saline. After 24 hrs of carbon-tetrachloride administration, rats were sacrificed. Blood was collected by retrorbital. The blood sample of each animal were taken and allowed to clot for 45 min. at room temperature. Serum was separated by centrifugation at 600 rpm for 15 min. and analyzed for various parameters. Hepatic tissues were removed and weighed accurately and homogenized in ice-cold 1.15% KCl to prepare 10% homogenate and centrifuged at 10,000 rpm for 60 min. The supernatant was used for assay of marker enzymes (GRD, GST, GSH and catalase).

2.3. *In-vivo* antioxidant activity

2.3.1. Lactate Dyhydrogenase(LDH)

The reaction mixture consisted of 0.1ml of NADH (0.02M) and 0.1ml of sodium pyruvate (0.01M) and 0.1 ml of serum and made up to 3ml. with sodium phosphate buffer (0.1M pH 7.4). The change in absorbance were measured at 340 nm at 30s interval each for 3 min. and the enzyme activity was calculated using molar extinction coefficient of 6.220/m/cm and was expressed as nanomoles NADH oxidized/min./mg protein [21].

2.3.2. Assessment of reduced glutathione (GSH) activity

The excised liver and kidney were rinsed with ice cold saline and blotted dry. They were homogenized in 5 volumes of 1% w/v picric acid. The homogenate was centrifuged. The supernatant was used for the assay. 0.2 ml of homogenate was added in the test tube and dissolved in 1.8-ml water. The solution was mixed thoroughly; 3 ml of precipitating solution was added promptly and mixed. Allowed to stand for 5 minutes in the room temperature and filtered through coarse filter paper then, the absorbance was measured at 412 nm the GSH concentration in the tissues was calculated and the results were expressed in U/g/min [21].

2.3.3. Glutathione-S-transferase(GST)

GST activity was measured by the method of Habig et al,(1974) [22]. The reaction mixture containing 1 ml of buffer, 0.1 ml of 1-chloro-2, 4-dinitrobenzene (CDNB), 0.1 ml of homogenate and 1.7 ml of distilled water was incubated at 37°C for 5 min. The reaction was then started by the addition of 1 ml of glutathione. The increase in absorbance was followed for 3 minutes at 340 nm. The reaction mixture without the enzyme was used as blank.

2.3.4. Catalase (CAT)

CAT was assayed by the method of Takahara et al, (1960) [23]. To 1.2 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the tissue homogenate was added and reaction was started by the addition of 1.0 ml of 30 mM H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30 s intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as μmoles of H2O2 decomposed/min/mg protein [21].

2.3.5. Glutathione Reductase (GRD)

The assay system consisted of 1.65ml of sodium phosphate buffer (0.1M, pH 7.4) and 0.1ml EDTA (0.5 Mm) and 0.05 ml oxidized glutathione (1Mm) and 0.1ml NADPH (0.1Mm) and 0.05ml supernatant in a total mixture of 2ml .The enzyme activity was quantified by measuring the disappearance of NADPH at 340nm at 30s interval for 3min.The activity was measured using molar extinction coefficient of 6.22x 10 3/m/cm and expressed as nanomoles NADPH oxidized/min./mg of protein.

2.4. Histopathological studies

For histological studies, the liver tissues were fixed in paraffin. Thin sections (5 mm) were cut and stained with routine hematoxylin and eosin (H & E) for photo-microscopic assessment (400×).The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue [24].

2.5. Statistical analysis

A one-way analysis of variance (ANOVA) followed by multiple comparisons with the Dunett-t- test was used to compare different parameters between the groups. A p value $P < 0.01$was considered significant.

3. RESULTS

3.1. Effect of *Bombax ceiba* on hepatic antioxidant enzymes

In an attempt to obtain more information on the mechanism of protection against CCl_4 hepatotoxicity by *Bombax ceiba*, we monitored the natural antioxidant cell defenses including the enzymes LDH, CAT, GRD, GST, and GSH in livers of animals. The activities of their tissue antioxidant enzymes decreased significantly in animals consequent to CCl_4 (Group II)- induced hepatic damage as compared to the normal animals (Group I). Oral administration of ethanolic leave and bark extract at the dose of 200mg/kg (Group IV/V) showed significant increase in LDH, CAT, GRD, GST and GSH when compared with CCl4-treated rats. Treatment with *Bombax ceiba* bark extract (Group V) showed only marginal increase in the level of antioxidant enzymes in liver homogenate compared with CCl4-treated rats (Table 1) but leave extract give acceptable activity. Thus, the activities of LDH, CAT, GRD, GST and GSH were restored by *Bombax ceiba* treatment.

Histopathological analysis of group I (Figure 1A) animals showed normal architecture. In rats treated with CCl_4 (Group II), the normal architecture of liver was

completely lost with the appearance of centrilobular necrosis. Scattered masses of necrotic tissues were detected in most of the areas in addition to the enlarged nuclei (Figure 1B). Treatment with *Bombax ceiba* (Groups IV and V) ameliorated the CCl4-induced liver injury and the typical histological changes were markedly alleviated in the liver sections (Figure 1D and E). Rats treated with silymarin (Group III) showed near normal architecture with uniform sinusoids (Figure 1C).

Table 1: Effect of ethanolic extract of *Bombax Ceiba* bark and leaves on tissues antioxidant parameters on CCl_4 Induced hepatotoxicity in rats

Groups n=6	Dose	LDH (nmol/min/lit)	GRD (mol/min/lit)	CAT (nmol/min/lit)	GSH (nmol/min/lit)	GST (µmol/min/lit)
I	Saline(2ml/kg)	342.19±0.87**	28.91±0.7**	185.15±0.24**	18.57±0.43**	5.06±0.08**
II	1(ml/kg)	552.18±0.43	47.06±0.54	46.85±0.18	34.05±0.4	15.03±0.49
III	10(mg/kg)	351.01±0.92**	22.39±0.36**	97.5±0.38**	23.53±0.28**	8.78±0.22**
IV	*Bombax ceiba* Leaf extract (200mg)	338.34±0.31**	19.48±1.09**	135.8±0.29**	24.73±0.48**	10.8±0.21**
V	*Bombax ceiba* Bark extract (200mg)	327.97±0.6**	18.04±0.19**	127.43±0.3**	28.55±0.99**	13.32±0.03**

*The Values are Mean ± SEM (n=6) ** P< 0.01*

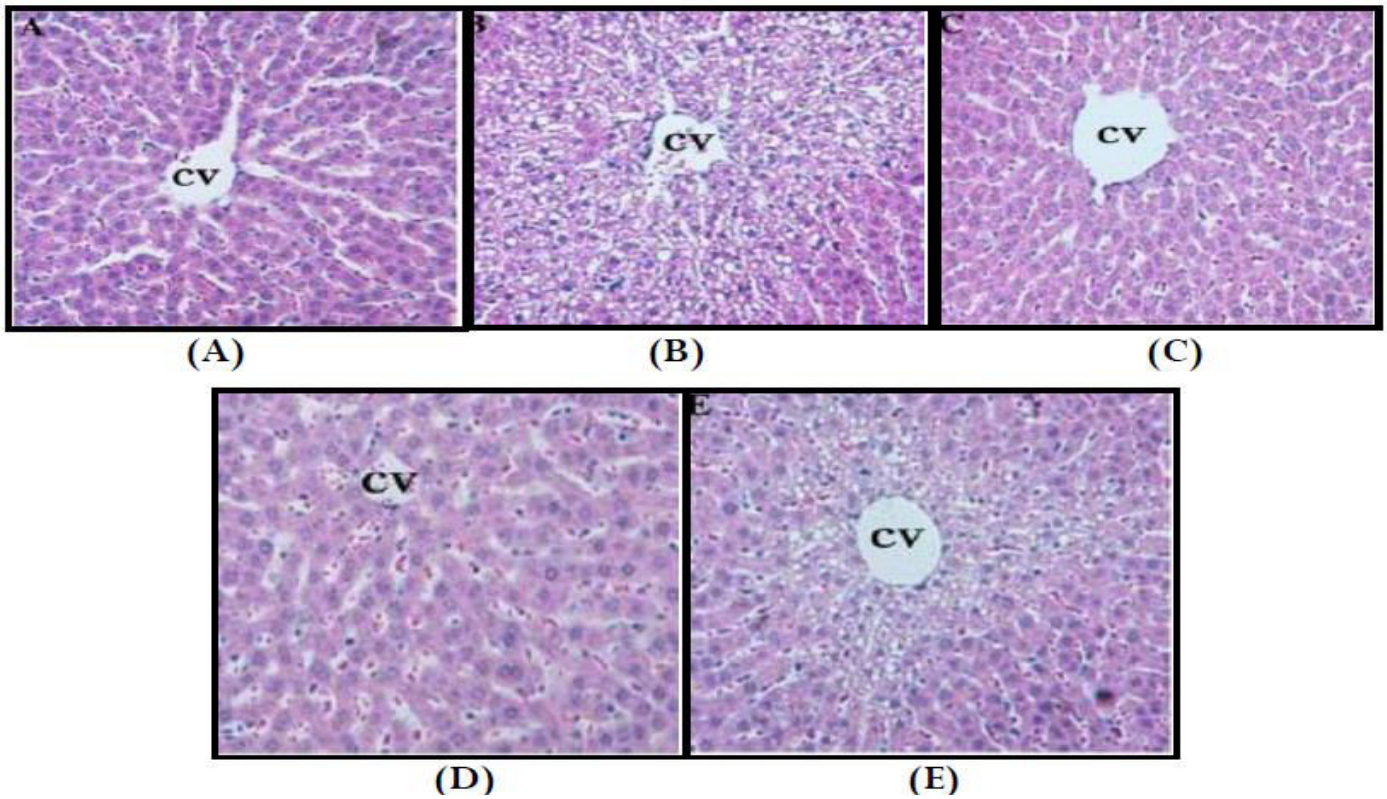

Fig. 1: Effect of *Bombax ceiba* on the histological morphology of rat liver by hematoxylin and eosin (H & E) staining, magnification, ×400. (A) Normal control, (B) CCl_4 control, (C) silymarin 10 mg/kg b.w.+CCl_4 (D) leave extract 200mg/kg b.w.+CCl_4 and (E) bark extract 200mg/kg b.w. + CCl_4

4. DISCUSSIONS

In *In-vivo* antioxidant study, CCl_4 induced hepatic damage may be due to its antioxidant capacity. It has been established that reactive oxygen species are involved in inflammation and protective action of plant extract against CCl_4 induced hepatic damage could involve mechanism related to scavenging activity [25]. In this study, rat treated with single dose of CCl_4 developed a

significant hepatic damage and oxidative stress, which was observed from a substantial increase in the activities of serum, LDH and CAT. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver [21]. Significant ($P<0.01$) increase in LDH, GRD, GSH, and GST activity was observed in CCl_4 treated group when compared with normal groups. The plant extract at 200mg/kg and standard drug, silymarin showed significant ($P<0.01$) reduction in LDH, GST, GRD, and GSH level when compared to CCl_4 treated and significant ($P<0.01$) reduction in Catalase activity was observed in CCl_4 treated rats when compared with normal group. The plant extract at 200mg/kg and standard drug, silymarin showed significant ($P<0.01$) increase CAT level when compared to CCl_4 treated. The present study revealed that all extracts reduced significantly the serum enzymatic activity. The increase in the level of serum transminase reflects liver damage as these enzymes are released in the blood circulation after the administration of hepatotoxin i.e. CCl_4. The toxicity is initiated by formation of a reactive metabolite trichlormethyl radical CCl_3 by microsomal fixed function oxidase. The activated CCl_3 radical binds covalently to the macromolecules and induces peroxidative degradation of membrane lipids resulting in hepatotoxicity and subsequent increase in serum transminase.

5. CONCLUSION

In the present study, it has been observed that *Bombax ceiba* herbal extract offered significant protection against the hepatotoxicant CCl_4.The marker enzyme levels and the important antioxidant enzymes activity were greatly protected, this plant is a superior remedy for any diseases of the liver. The study also shows that plant extract has significant antioxidant property. Further isolation of active principles will be advantageous to produce novel bioactive constituents from these extracts, which may possess more significance in the treatment of liver diseases, and to elucidate its exact mechanism of action.

6. REFERENCES

1. King PD, Perry MC. *The Oncologist*, 2001; **6:**162-176.
2. DeBethizy JD, Hayes JR. Metabolism: a determinant of toxicity. In Hayes AW (Ed) Principles and Methods of Toxicology. Philadelphia: Taylor and Francis: 2001, p.77-136.
3. Loguercio C, Federico A. *Free Radic Biol Med*, 2003; **34(1):**1-10.
4. Vitaglione P, Morisco F, Caporaso N, Fogliano V. *Crit Rev Food Sci Nutr*, 2004; **44:**575-586.
5. Handa SS, Sharma A, Chakravarti KK. *Fitotherapia*, 1986; **57:**307-312.
6. Subrata D, Ravishankar B, Bhavsar GC. *Indian Drugs*,1994; **30(8):**355-363.
7. Hensley K, Robinson K, Gabbita SP, Salsman S, Floyd RA. *Free Radic Biol Med*, 2000; **28:**1456-1462.
8. Kaplowitz N. *Semin Liver Dis*, 2002; **22:**137-144.
9. Higuchi H, Gores GJ. *Curr Mol Med*, 2003; **3:**483-490.
10. Mc Clements J, Decker EA. *J Food Sci*, 2000; **65:**1270-1282.
11. Singh N, Kamath V, Narasimhamurthy K, Rajini PS. *Environ Toxicol Pharmacol*, 2008; **26(2):**141-146.
12. Nadkarni KM. Indian Materia Medica. Popular Prakashan 1976; p. 43.
13. Varier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants-A Compendium of 200 species. Orient Longman Publishing, Kerala 1997; p. 289-292.
14. Warrier PK. Indian Medicinal Plants a compendium of 200 species, Vol. 2, Orient Longman private limited Chennai 1994; p. 224-289.
15. Buckingham J. Dictionary of Natural Products. Landon: Champan and Hall Scientific Data Division 1992. p. 36-38.
16. Saleem R, Ahmad SI, Ahmad M, et al. *Biol Pharm Bull,* 2003; **26:**41-46.
17. Saleem R, Ahmad M, Hussain SA, et al. *Planta Med*, 1999; **65(4):**331-334.
18. Vieira TO, Said A, Aboutabl E, et al. *Redox Report,* 1999; **146:**41-46.
19. Rastogi RP, Mehrotra BN, Compendium of Indian Medicinal Plants, Vol. 2, PID, New Dehli, 1990; p.104.
20. Badria FA, Mikhaeil BR, Maatooq GT, Amer MM. *Z Naturforsch C J Biosci*, 2003;**58(7-8):**505-516.
21. Mukherjee PK. Quality control of herbal drugs. 2nd Ed. Business Horizons; 2007.
22. Habig WH, Pabst MJ, Jakoby WB. *J Biol Chem*, 1974; **249:**7130-7139.
23. Takahara S, Hamilton BH, Nell JV, Ogubra TY, Nishimura ET. *J Clin Invest*, 1960; **39:**610-619.
24. Amresh G, Rao Ch V, Singh PN. *Nutr Res*, 2007; **27:**625-632.
25. Jin YS, Sa JH, Shim TH, Rhee HI, Wang MH. *Biochem Biophys Res Commun*, 2005; **329(3):**991-995.