www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Evaluation of Antioxidant and Hepatoprotective effects of 70 % ethanolic bark extract of *Albizzia lebbeck* in rats

Tushar Patel*[1], Devendra Shirode[2], Samaresh Pal Roy[2], Sunil Kumar[2], S Ramachandra Setty[2]

[1]Dept. of Pharmacology, Sat Kaival College of Pharmacy, Sarsa, Gujarat, India
[2]Dept. of Pharmacology, S.C.S. College of Pharmacy, Harapanahalli, Karnataka, India

ABSTRACT

The Plant *Albizzia lebbeck* is reported to contain polyphenolic compounds. However, there are no reports on the antioxidant and organ protective properties of this plant. Hence, the present study was planned with an objective to evaluate the plant for its antioxidant (both *in vitro* and *in vivo*) and hepatoprotective properties against CCl_4 induced hepatotoxicity in rats. The antioxidant activity was evaluated by using various *in-vitro* models like reducing power, superoxide anion scavenging and hydroxyl ion scavenging activity. The hepatoprotective activity was assessed by using CCl_4 induced hepatotoxicity in rats. Concentrations of various biochemical markers like SGPT, SGOT, Total and Direct Bilirubin, ALP, tissue GSH, lipid peroxidation and various physical parameters were estimated to determine the extent of hepatic damage. In addition histopathological observation was also made so as to assess the organ protective potential of the test extract. The test plant has shown dose dependant antioxidant activity in all the models of the study. Pretreatment with test extract (200mg/kg and 400mg/kg) prevented the depletion of tissue GSH, lipid peroxidation and reduced the elevated levels of all the biochemical markers of hepatotoxicity, indicating that the test extract possess hepatoprotective property. The histopathological study exhibited near to normal liver architecture as compared to control. The result of present study suggests that 70% ethanolic extract of bark of *Albizzia lebbeck* possesses Antioxidant and Hepatoprotective effects in rats.

Keywords: *Albizzia lebbeck;* Hepatoprotective; Antioxidant; CCl_4.

INTRODUCTION

The term 'exogenous antioxidant' refers to numerous vitamins, minerals and other phytochemicals to protect against the damage caused by reactive oxygen species (ROS). The ROS such as superoxide anion radical, hydrogen peroxide and hydroxyl radical have been implicated in the pathophysiology of various clinical disorders, including aging (Finkel et al., 2000), cancer (Hemnani et al., 1998) and atherosclerosis (Ross, 1999). These are highly reactive species and capable of damaging nucleus and cell membranes by reacting with various vital intracellular molecules like DNA, protein, carbohydrates and lipids (Young et al., 2001). Free radicals and other reactive oxygen species are derived either from normal metabolic process in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air-pollutants and industrial chemicals (Kelly et al., 1995). The inhibition/quenching of free radicals can serve as facile model for evaluating the activity of hepatoprotective agents.

Liver disease is a worldwide problem. No reliable drugs are available in allopathic medical practice. Therefore, there is a worldwide trend to go back to traditional medicinal plants. Hence, herbs play a major role in the treatment of liver disease. However, search for herbs available at hands stretch for treating hepatitis is continuing to reduce the cost of treatment. In continuation with the trend, we in our institution have started for searching the herbs that are useful in hepatitis. In one of our field survey, the plant by name *Albizzia lebbeck* known as Siris was found and upon literature survey, it was found that the plant contains tannins and polyphenolic compounds (Arvind et al., 2007). There are reports that the polyphenolic compounds are possessing anti-oxidant and organ protective properties (Tiwari, 2001). Hence, this plant was selected for the present study.

The modern literature revealed that the plant is reported to possess nootropic (Chintawar et al., 2002), anxiolytic (Une et al., 2001), anticonvulsant (Kasture et al., 2000), antifertility (Gupta et al., 2004), antidiarrhoeal (Besra et al., 2002) and anti-inflammatory activity (Pramanik et al., 2005). The present study was undertaken to study the possible hepatoprotective and antioxidant role of 70% Ethanolic extract of bark of *Albizzia lebbeck* (EEBAL).

* Corresponding Author
Email: tush9_pharmacist@yahoo.com
Contact: +91-9974077638
Received on: 27-04-2010
Revised on: 29-06-2010
Accepted on: 07-07-2010

MATERIALS AND METHODS

Plant Material

The bark of plant *Albizzia lebbeck* was collected from fields of Harapanahalli, Karnataka in the month of May 2007. It was identified and authenticated by Prof. K.Prabhu, H.O.D., Dept of Pharmacognosy, S.C.S. College of Pharmacy. A herbarium specimen (SCS-07-12) was preserved in the college herbal museum for future reference.

Preparation of 70% EEBAL

The bark was shade dried at room temperature and pulverized. The 70% ethanolic extract of bark of *Albizzia lebbeck* was prepared by using 70% ethanol in a soxhlet apparatus after de-fatting with petroleum ether. Preliminary phytochemical investigation showed the presence of 7-11% tannins, flavanoids and saponins in 70% EEBAL. So 70% EEBAL was selected for the present activity.

Experimental animals and feeds

Albino Wistar rats (150-250g) and mice (25-35g) of either sex were obtained from animal house of Venkateshwara Enterprise, Bangalore, Karnataka, India. All the animals were housed in a room maintained at 22 ± 1°C with a relative humidity of 60 ± 5% and a 12-hr light-dark cycle. They were allowed to acclimatize for a week prior to experiment and had free access to standard pellet diet (Hindustan Lever Pellets, Bangalore, India), water was provided *ad libitum*. All experiments were carried out with strict adherence to ethical guidelines and were conducted as per approved protocol by the Institutional Animal Ethics Committee (Reg. no.157/1999/CPCSEA) and as per Indian norms laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

Acute Toxicity studies

The acute toxicity was determined on albino mice by fixed dose method of OECD Guide line no 420 given by CPCSEA (Veeraraghavan, 2000). Groups of six mice were administered test drug by oral route in the range of 2000-300 mg/kg and mortality was observed after 24 hr.

Reducing power activity

The reducing power of 70% EEBAL was determined according to the method of Oyaizu (Oyaizu, 1986). Different doses of 70% EEBAL were mixed in 1 ml of distilled water to get 20μg-100μg concentration. This was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The percentage increase in reducing power was calculated by using the following formula:

$$\% \text{ increase in absorbance} = \frac{\text{Test OD} - \text{Control OD}}{\text{Control OD}} \times 100$$

Superoxide anion scavenging activity

Measurement of Superoxide anion scavenging activity of 70% EEBAL was done by using the method explained by Nishimiki (Nishimiki et al., 1972) and modified by Ilhams et al., 2002. About 1 ml of Nitroblue Tetrazolium (NBT) solution (156μM of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of various concentrations of 70% EEBAL and standard in water was mixed. The reaction was started by adding 100 μl of Phenazine Methosulphate (PMS) solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance at 560 nm was measured against blank samples. Decrease in absorbance of the reaction mixture indicated increase superoxide anion scavenging activity. The activity was measured in triplicate and mean result was taken. The antioxidant activity was expressed in terms of percentage inhibition (micromole or microgram/ ml concentration required to inhibit phenyl hydrazine and PMS radical formation). The % inhibition was calculated by using the following formula:

$$\% \text{ inhibition in absorbance} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Hydroxyl radical scavenging activity

Hydroxyl radical generation by phenyl hydrazine has been measured by the 2-deoxyribose degradation assay of Hathwell and Gutteridge, 1981. In 50mM phosphate buffer (pH 7.4), 1mM deoxyribose, 0.2mM phenyl hydrazine hydrochloride were prepared. 0.6ml of 1mM deoxyribose and 0.4ml of various concentrations of 70% EEBAL and standard were mixed. 0.6 ml phosphate buffer was added to make reaction solution 1.6ml. After 10 min incubation at 37^0C, 0.4ml of 0.2 mM phenyl hydrazine was added. Incubation was terminated after 4 hrs and 1 ml each of 2.8% TCA and 1 ml of 1% (w/v) thiobarbituric acid were added to the reaction mixture and heated for 20 mins in a boiling water bath. The tubes were cooled to room temperature and absorbance was measured at 532 nm. The percentage reduction in the OD is calculated by using the earlier formula.

CCl_4 induced hepatotoxicity

Rats were divided into five groups of six animals each. Group-I and Group II received distilled water (1ml/ kg) for 5 days. Group III received 100 mg / kg silymarin

Table 1: In-vitro antioxidant effect of 70% EEBAL

Treatment	Reducing power Mean ± SEM (% increase)	Superoxide anion Scavenging Mean ± SEM (% inhibition)	Hydroxyl radical scavenging Mean ± SEM (% inhibition)
Control	0.295±0.0023	0.455 ±0.0033	0.307 ± 0.0005
Standard 25 µg	0.560±0.0017*** (89.83%)	0.033±0.0035*** (92.74%)	0.134 ± 0.0015*** (56.35%)
70% EEBAL 20 µg	0.304±0.0028*** (03.05%)	0.353±0.0026*** (22.41%)	0.264 ± 0.0056*** (14.00%)
70% EEBAL 40 µg	0.399±0.0028*** (35.25%)	0.235±0.0046*** (48.35%)	0.250 ± 0.0024*** (18.56%)
70%.EEBAL 60 µg	0.419±0.0037*** (42.03%)	0.202±0.0025*** (55.60%)	0.232 ± 0.0041*** (24.42%)
70% EEBAL 80 µg	0.539±0.0003*** (82.71%)	0.153±0.0025*** (66.37%)	0.202 ± 0.0056*** (34.20%)
70% EEBAL100 µg	0.728±0.0014*** (146.7%)	0.114±0.0036*** (74.94%)	0.175 ± 0.0032*** (42.99%)

Values are the mean ± S.E.M., n=3**;** standard: Sodium metabisulphate
Significance *** P<0.001 compared to control.

Table 2: Effect of 70% EEBAL on tissue GSH and Lipid peroxidation levels in CCl4 induced hepatotoxicity

Treatment	Tissue GSH level		Tissue lipid peroxidation	
	Mean ± SEM	% Increase	Mean ± SEM	%Inhibition
Negative control (1ml dist. Water p.o.)	0.960 ± 0.03	--	0.232 ± 0.018	--
CCl_4 (positive control) (2ml/kg s.c.)	0.486 ± 0.025	--	0.455 ± 0.028	--
CCl_4+ Silymarin (2ml/kg s.c + 100 mg/kg, p.o.)	0.951 ± 0.026***	95.88%	0.170 ± 0.023***	62.64%
CCl_4+70% ethanolic extract (2ml/kg s.c. + 200 mg/kg p.o.)	0.647 ± 0.027***	35.39%	0.298 ± 0.015***	34.50%
CCl_4+70% ethanolic extract (2ml/kg s.c.. + 400 mg/kg p.o.)	0.793 ± 0.021***	73.25%	0.206 ± 0.028***	54.62%

Values are the mean ± S.E.M., n=6
Significance *** P<0.001 compared to CCl_4 control

(standard drug) orally for 5 days. Group IV and Group V received 200 mg/kg and 400 mg /kg 70% EEBAL (orally) respectively for 5 days. Group-I received liquid paraffin (1 ml/kg) s.c., Group-II, III, IV and V received CCl_4: liquid paraffin (1:1) at a dose of 2 ml/kg s.c., on days 2 and 3, after 30 min of vehicle, 100 mg/kg silymarin, 200 mg/kg and 400 mg/kg of 70% EEBAL administration. Food was withdrawn 12 hr before carbon tetrachloride administration to enhance the acute liver damage in groups 2, 3, 4 and 5. On the fifth day, all the animals were sacrificed by mild ether anaesthesia. Blood samples were collected for evaluating the biochemical parameters and liver tissue samples were collected for histological studies (Suja et al., 2004).

In vivo tissue Glutathione estimation

Tissue Glutathione measurements were performed using a modification of Ellamn procedure (Aykae et.al., 1985). Liver tissue samples were homogenized in ice-cold trichloroacetic acid (1gm tissue in 10 ml 10% TCA) in an ultra turrax tissue homogenizer. The mixture was centrifuged at 3000 rpm for 10 min. Then 0.5 ml of supernatant was added to 2 ml of (0.3M) disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium acetate) was added and absorbance was taken at 412 nm immediately after mixing. Percentage increase in the OD is directly proportional to the increase in the level of glutathione. Hence % increase in OD was calculated.

In vivo lipid peroxidation estimation

The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation (John Buege et al., 1978). Combine 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0 µmol of lipid phosphate) with 2.0 ml of TCA-TBA-HCL and mixed thoroughly. Solution was heated for 15 min and cooled. The precipitate was removed by centrifugation at 1000 rpm for 10 min and absorbance of sample was determined at 535 nm against a blank that contains all the reagents minus lipid.

Table 3: Effects of 70% EEBAL on Physical parameters and Biochemical markers in CCl4 induced hepatotoxicity

Treatment	Liver volume (ml/100g)	Wet liver weight (g/100g)	Biochemical parameters Mean ± SEM				
			SGOT IU/L	SGPT IU/L	ALP IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
Negative control	3.6 ± 0.1450	3.09 ± 0.1167	147.85 ± 2.761	53.89 ± 2.053	144.53 ± 3.577	0.89 ± 0.017	0.18 ± 0.018
Positive Control	5.2 ± 0.2509	4.75 ± 0.2566	415.01 ± 2.418	330.51 ± 5.894	449.03 ± 15.619	3.42 ± 0.019	1.63 ± 0.063
CCl_4+ Silymarin	3.72 ± 0.11***	3.55 ± 0.16**	165.7 ± 3.784***	56.83 ± 2.993***	187.2 ± 6.813***	1.00 ± 0.057***	0.256 ± 0.014***
CCl_4 +70% ethanolic extract (200mg/Kg)	3.9 ± 0.23***	3.8 ± 0.30*	226.7 ± 9.048***	78.78 ± 1.565***	311.6 ± 15.462***	2.84 ± 0.086*	1.30 ± 0.031***
CCl_4 +70% ethanolic extract (400mg/Kg)	3.76 ± 0.14***	3.53 ± 0.12**	177.6 ± 4.640***	67.04 ± 4.217***	225.8 ± 7.764***	1.27 ± 0.057***	0.52 ± 0.051***

Values are the mean ± S.E.M, n = 6

Significance ^{ns}P>0.05, **P <0.01 and *** P<0.001 compared to CCl_4 treatment.

Biochemical estimations

Blood was obtained from all the animals by puncturing retro-orbital plexus. Collected blood samples was centrifuged (2000 rpm for 10 mins) to get clear serum and was used to estimate various biochemical parameters like serum enzymes: SGPT (Bradley et al., 2003), SGOT (Rej et al., 1973), ALP (McComb et al., 1972), Bilirubin (Pearlman et al., 1974).

Histopathological studies

The liver was excised from the animals and washed with the normal saline. Seven micrometer thick paraffin sections of buffered formalin-fixed liver samples were stained with haematoxylin-eosin for photomicroscopic observations of the liver histological architecture of the control and treated rats.

Statistical analysis

Experimental results were expressed as mean ± SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Turkey-Kramer multiple comparisons test. P value less than 0.05 & 0.001 was considered to be statistically significant (p<0.05, p<0.001).

RESULTS

Acute toxicity

An attempt was made to determine LD_{50} of 70% ethanolic extract of *Albizzia lebbeck* bark. Since no mortality was observed at 2000 mg/kg, it was thought that 2000 mg/kg was the cut off dose. Therefore 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of cut off dose were selected for further study i.e. for screening hepatoprotective property.

Antioxidant activity

In all models, 70% EEBAL showed dose dependent antioxidant activity (table 1). The 70% EEBAL showed 146.7 % reducing power, 74.94 % super oxide anion and 42.99 % hydroxyl radical scavenging activities at 100µg concentration, which are comparable to that of Sodium metabisulfate 25 µg (Table 1).

In vivo GSH

There was a marked depletion of GSH level in CCl_4 treated group. Silymarin 100 mg/kg increased tissue GSH by 95.88%. Treatment with 70% ethanolic extracts showed a dose dependent increase in the levels of GSH. However, both doses of 70% EEBAL have shown lesser increase GSH level than standard Silymarin (Table 2).

In vivo lipid peroxidation

CCl_4 has enhanced the lipid peroxidation. The treatment with 70% EEBAL has significantly reduced the lipid per-oxidation in a dose dependant manner. Silymarin 100 mg/kg showed 62.64 % inhibition, whereas 400 mg/kg of 70% EEBAL showed 54.62 % inhibition, which was almost near to standard Silymarin (Table 2).

CCl_4 induced hepatotoxicity

Increased levels of liver weight (4.75 gm/100gm), liver volume (5.2 ml/100gm), SGPT (330.51 IU/l), SGOT (415.01 IU/l), ALP (449.03 IU/l), Total Bilirubin (3.42

mg/dl) and Direct Bilirubin (1.63 mg/dl) observed in CCl_4 treated group. The pretreatment with 70% EEBAL (200 mg/kg and 400 mg/kg p.o.) has brought back the elevated levels of biomarker enzymes of hepatitis in a dose dependant manner. Treatment with 400 mg/kg of EEBAL has produced the hepatoprotective activity comparable to that of Silymarin 100 mg/kg p.o. (Table 3).

Histopathology

CCl_4 treatment has altered the liver architecture as indicated by the histopathological observations that showing extensive fatty change more around central vein with micro-vesiculisation and fatty change. Liver sinusoids were also congested. However, treatment with 70% EEBAL has shown dose dependant improvement in the liver architecture as indicated by the histopathological observations that there was mild inflammation, slight congestion and fatty change to a lesser extent. The reversal of wet liver weight, volume, levels of biochemical markers and histopathological observations reveal that the 70% EEBAL possess hepatoprotective activity against CCl_4 induced hepatotoxicity in albino rats.

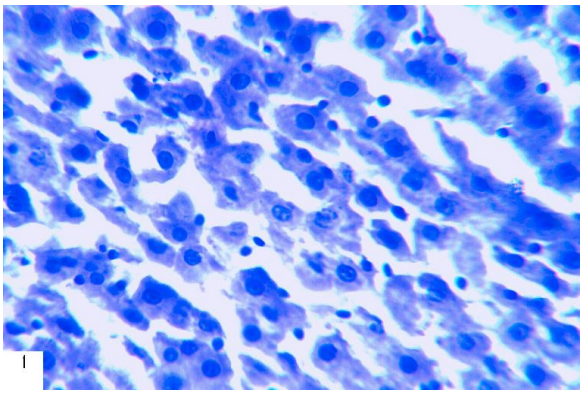

Figure 1: Liver section of normal control rat showing hepatic cells with nuclei, cytoplasm, normal central vein and portal vein with no inflammation (100x)

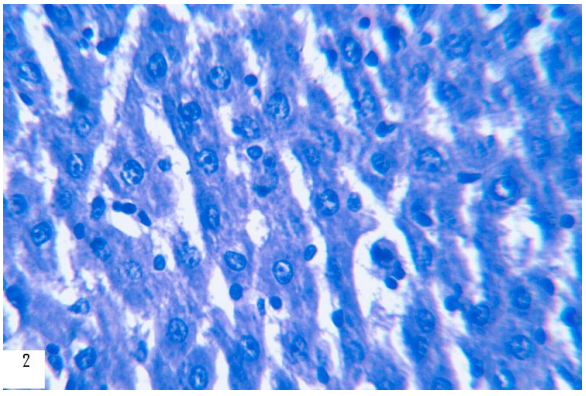

Figure 2: Liver section of CCl4 treated rat showing marked necrosis, severe fatty generation and extensive vacoulisation (100x)

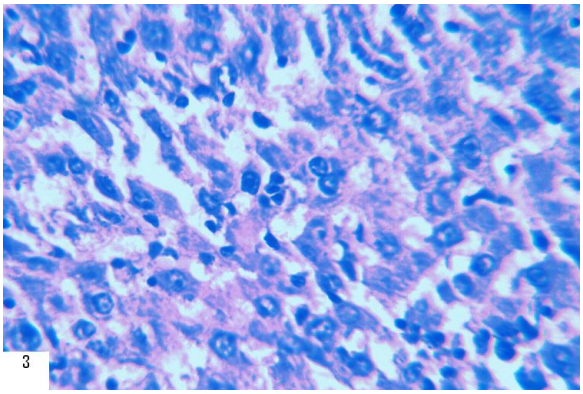

Figure 3: Liver section of Silymarin + CCl4 treated rat showing normalcy of hepatic cells (100x)

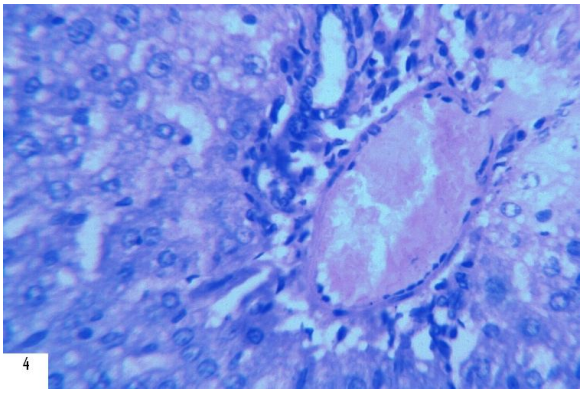

Figure 4: Liver section of 200 mg/kg EEBAL + CCl4 treated rat showing mild peroportal inflammation and fatty change with mild congestion (100x)

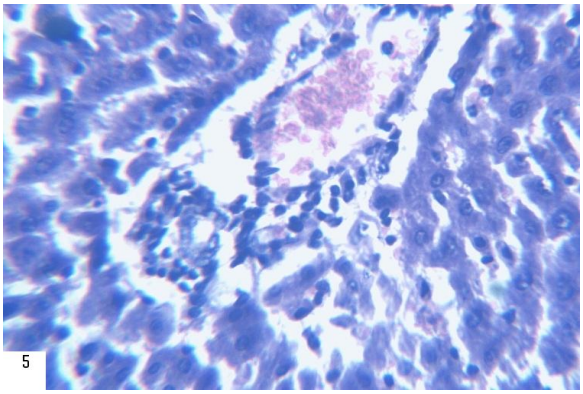

Figure 5: Liver section of 400 mg/kg EEBAL + CCl4 treated rat showing marked improvement over CCl4 control group (100x)

DISCUSSION

The antioxidant activity of 70% EEBAL was assessed on the basis of reducing power, superoxide anion and hydroxyl radical scavenging activity (in vitro antioxidant models) & effect on tissue GSH and lipid peroxidation (in vivo antioxidant model). The 70% EEBAL showed dose dependant reducing power, superoxide anion and hydroxyl radical scavenging activities. Subcutaneous administration of CCl_4 for two days elevated the SGPT,

SGOT, ALP, total and direct bilirubin. These findings in positive control are in conformity with the earlier reports. Pretreatment with 70% ethanolic extract (200 mg/kg and 400 mg/kg p.o.) for 5 days significantly reduced the elevated biochemical markers in a dose dependent manner. Treatment with 400 mg/kg of 70% EEBAL produced the hepatoprotective activity comparable to that of silymarin 100 mg/kg p.o. CCl_4 is metabolized to trichloromethyl $CCl_3^{\bullet}$ radical due to the catalytic activity of CYP 450 $2E_1$ enzyme, which is further converted to trichloromethyl peroxy radical by superoxide anions. This trichloro methyl peroxy radical is the main culprit in causing hepatotoxicity. This particular radical forms a covalent bond with sulphydryl group of membrane GSH, protein thiols and unsaturated fats or lipids. This covalent bonding of free radicals with cellular macromolecules initiates the cascade of reactions leadings to lipid peroxidation (Kyung Jin Lee et al., 2004, Be-Jen Wang et al., 2004). The lipid peroxidation inturn alter the membrane permeability and initiates chain of reaction leading to tissue damage and necrosis. It was observed that the test extract has shown significant reducing power and superoxide anion scavenging activity. Therefore pretreatment with 70% EEBAL may be preventing the formation of trichloro methyl peroxy radical due to superoxide anion scavenging activity. Thereby tissue GSH levels are not depleted and lipid peroxidation is minimized, this may be the possible mechanism of hepatoprotection offered 70% EEBAL. However our studies do not confirm whether test extract block CYP 450 $2E_1$ enzyme and thereby inhibit the formation trichloromethyl $CCl_3^{\bullet}$ radical.

CONCLUSION

The present study demonstrates that 70% EEBAL possesses antioxidant and hepatoprotective activity. In addition, the hepatoprotective property may be attributed to the antioxidant principles of plant, namely tannins and flavonoids. Further investigation is going on to isolate, characterize and screen the active principles that possess antioxidant and hepatoprotective property.

ACKNOWLEDGEMENTS

The authors thank Sri. Sha. Bra. Chandramouleshwara Swamiji, the President and Sri. T. M. Chandrashekharaiah, the Secretary, T.M.A.E society for providing all the facilities to carry out this research work, Dr. Shashikala P., HOD, Department of Pathology, SSIMS, Davangere for her support in histopathological studies and Microlabs, Bangalore for providing standard drug (Silymarin).

REFERENCES

Finkel T, Holbrok NJ. 'Oxidant, oxidative stress and the biology of aging Nature', Vol. 408, 2000 pp.239-247.

Hemnani T, Parihar MS. 'Reactive oxygen species and oxidative DNA damage', Indian Journal of Physiology and Pharmacology, Vol. 42, 1998 pp. 440-452.

Ross R. 'Atherosclerosis: An inflammatory disease', N Eng J Med, Vol. 340, 1999 pp. 115-126.

Young IS, Woodside JV. 'Antioxidant in health and disease'. J Clin. Pathol. Vol. 54, 2001 pp. 176-186.

Kelly FJ, Mudway MT, Krishna MT. 'The free radical bases of air pollution focus on ozone', Respir. Med. Vol. 89, 1995 pp.647-656.

Asolkar LV, Kakkar KK. 'Second Supplement to Glossary of Indian Medicinal Plants with active principles, New Delhi: C.S.I.R. Publications; 1965-81: 36-38.

Arvind K, Saluja AK, Shah UD, Mayavanshi AV. 'Pharmacological potential of *Albizzia lebbeck*. Review', Vol.1, 2007 pp.171-174.

Tiwari K. Ashok. 'Imbalance in antioxidant defence and human disease', Curr Sci, Vol. 81, 2001 pp. 1179-1186.

Chintawar SD, Somani RS, Kasture VS, Kasture SB. 'Nootropic activity of *Albizzia Lebbeck* in mice', Journal of Ethanopharmacology, Vol. 81, 2002 pp. 299-305.

Une HD, Sareiya VP, Pal SC, Kasture VS, Kasture SB. 'Nootropic and anxiolytic activity of saponin of *Albizzia lebbeck* leaves', Pharmacology Biochemistry and Behavior, vol. 69, 2001 pp. 439-444.

Kasture VS, Kasture SB, Pal SC. 'Anticonvulsant activity of *Albizzia lebbeck* leaves', Indian J of Exp. Biol., vol. 34, 1996 pp. 78-80.

Kasture VS, Chopade CT, Deshmukh VK. 'Anticonvulsant activity of *Albizzia lebbeck*, *Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals', Journal of Ethanopharmacology, vol. 71, 2000 pp. 65-75.

Gupta RS, Kachhawa JB, Chaudhary R. 'Antifertility effects of methanol pod extract of *Albizzia lebbeck (L)* Benth in male rats', Asian J Androl., vol. 6, 2004 pp. 155-159.

Besra SE, Gomes A, Chaudhury L, Vedasiromoni JR, Ganguly DK. 'Antidiarrhoeal activity of seed extract of *Albizzia lebbeck* Benth', Phytotherapy Res., vol. 16, 2002 pp. 529-533.

Pramanik K.C., Bhattacharya P., Chatterjee T.K., Mandal S.C., 'European Bulletin of drug research', vol. 13**,** 2005 pp. 71-75.

Veeraraghavan P. Expert consultant, 'CPCSEA, OECD guideline No. 420', Oct. 2000.

Oyaizu M. 'Studies on product of browning reaction preparation from glucose amine', Jap J Nutrition, vol. 44, 1986 pp.307-312.

Ilhams Gulcin, Munir Oktay, Irfan Kufre Vioglu O, Ali Aslan. 'Determinations of antioxidant activity of lichen *Cetraria islandica* (L) Ach', J. Ethanopharmacol, vol. 79 2002 pp. 325-329.

Barry Hathwell, John Gutteridge MC. 'Formation of a thiobarbituric acid reactive substance from deoxyribose in the presence of Iron salts', FEBS Letters, vol.128, no. 2 1981 pp. 347-352.

Suja SR, Latha PG, Pushpangadan P, Rajasekharan S.'Evaluation of hepatoprotective effects of *Helminthostachyes zeylanica* (L.) Hook against carbon tetrachloride-induced liver damage in Wister rats', J. Ethnopharmacol, vol. 92, 2004 pp. 61-66.

Bradley DW, Maynard JE, Emery G, Webster H. 'Transminase activity in serum of long term hemolysis patients', Clin. chem., vol. 18, 2003 pp. 1442.

Rej R, Fasce CF, Vanderlinde RE. 'Increased aspartase aminotransferase activity of serum after in vitro supplymentation with pyridoxal phosphate', Clin. Chem., Vol. 19, 1973 pp. 92.

McComb RB, Bowers GN. Jr. 'Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum', Clin. Chem., vol. 18, 1972 pp.97.

Pearlman PC, Lee RT. 'Detection of measurement of total Bilirubin in serum with use of surfactants as solubilizing agents', Clin. Chem., vol. 20, 1974 pp. 447.

Aykae G, Vysal M, Yalein AS, Kocak TN, Sivas A, Oz H. 'The effect of chronic ethanol ingestion on hepatic lipid peroxide, Glutathione, glutathione peroxidase and glutathione transferase in rats', Toxicology, vol. 36, 1985 pp. 71-6.

John Buege A, Steven Aust D. 'Microsomal lipid peroxidation', London: Moury Kleiman Co.; 1978 pp. 302.

Kyung Jin Lee, Eun-Rhan Woo, Chul Yung Choi, Dong Weon Shin, Dong Gun Lee, Ho Jin You. 'Protective effect of Acteoside on carbon tetrachloride-induced hepatotoxicity', Life Sciences, vol. 74, 2004 pp. 1051-1064.

Hye Gwang Jeong. 'Inhibiton of cytochrome P450 2E1 expression by oleanolic acid: hepatoprotective effects against carbon tetrachloride-induced hepatic injury', Toxicol Letter, vol. 105, 1999 pp. 215-222.

Be-Jen Wang, Chu-ting Liu, Chin-Yin Tseng, Chien-Ping Wu. 'Hepatoprotective and antioxidant effects of *Bupleurum kaoi Liu* (Chao et Chuang) extract and its fractions fractionated using supercritical CO_2 on CCl_4-induced liver damage', Food and Chemical Toxicology, vol. 42, 2004 pp. 609-617.