Hepatoprotective activity of LIV-first against carbon tetra chloride-induced hepatotoxicity in albino rats

T. B. Lima, A. Suja, O. S. Jisa, S. Sathyanarayanan, K. S. Remya
Amrita School of Pharmacy, Amrita Viswavidhyapeedham University, Elammakkara P.O., Kochi - 682 026, India

Liver toxicity is a major health problem of worldwide proportions. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. In the present study, LIV-first (16.3 mg/kg, *p.o.*) was used to screen the hepatoprotective activity. Hepatotoxicity was induced in experimental animals by administration of carbon tetrachloride (CCl_4) (1 ml/kg, *i.p*). Silymarin (25 mg/kg,*p.o.)* was used as the standard. Biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin were measured. Cytotoxicity of CCl_4 was estimated by quantitating the release of malondialdehyde. The activity of tissue antioxidant enzymes namely superoxide dismutase, catalase and the level of total protein and glutathione were also measured. Histopathological evaluation of liver sections was also done. CCl_4 administration in rats elevated the levels of SGPT, SGOT, ALP and bilirubin. Administration of LIV-first significantly ($P<0.01$) prevented this increase. The activity of anti-oxidant enzymes in carbon tetrachloride CCl_4-treated group was decreased and these enzyme levels were significantly ($P<0.05$) increased in LIV-first-treated groups. Histopathological studies revealed that the concurrent administration of CCl_4 with the extract exhibited protection of the liver tissue, which further evidenced the above results. The study confirmed the hepatoprotective activity of LIV-first, which may be attributed to its antioxidant property.

Key words: Hepatoprotective activity, LIV- first Albino rats, carbon tetrachloride, LIV- first, silymarin

INTRODUCTION

The liver performs many functions vital to the health of the organism. The liver transforms and excretes many drugs and toxins. These substances are frequently converted to inactive forms by reactions that occur in the hepatocytes.[1-3] Historically, plants have been used in the folk medicine to treat various diseases. Experimental work on several plants has been carried out to evaluate their efficacy against chemically induced liver toxicity.

Carbon tetrachloride (CCl_4) was the first toxin for which it was shown that the injury it produces is largely or entirely mediated by a free-radical mechanism. Its main toxic effects are shown on the liver. Toxic levels administered to animals produce fatty accumulation in the liver due to a blockage in the synthesis of the lipoproteins that carry triglycerides away from this organ. It is believed that CCl_4 is metabolized by the P_{450} system to give the trichloromethyl radical, a carbon-centred radical. Several P_{450} are involved including CYP2E1, the 'ethanol-inducible' cytochrome P_{450}. Hence, CCl_4-induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver.[2,4]

LIV-first is a polyherbal Ayurvedic proprietary medicine which is used as a hepatoprotective agent. Each capsule contains *Eclipta alba* (100 g), *Andrographis paniculata* (50 mg), *Tinospora cordifolia* (100 mg), *Picrorhiza kurroa* (75 mg), *Boerhavia diffusa* (50 mg) and *Berberis aristata* (50 mg). The present study was to investigate the hepatoprotective activity of LIV-first against CCl_4-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

Adult Wistar male albino rats weighing between 150 and 200 g were used for the study. They were kept under standard laboratory conditions and were fed with commercial rat pellets and drinking water *ad libitum*. The animals were housed in polypropylene cages. Ethical committee in accordance with animal experimentation and care has approved all animal procedures.

Drugs and Chemicals

LIV-first capsule	Nectur Herbal Research Laboratories, Tamil Nadu
Carbon tetrachloride	SD Fine Chemicals, Mumbai
Silymarin	Microlabs, Bangalore
Reduced glutathione	HiMedia Laboratories Pvt. Ltd, Mumbai

Address for correspondence: Dr. Lima Trisa Baby, Amrita School of Pharmacy, Amrita Viswavidhyapeedham University, Elammakkara P.O., Kochi - 682 026, India. E-mail: limatrisa@gmail.com
Received: 23-09-2008; **Accepted:** 20-11-2008; DOI: 10.4103/0973-8258.63878

All drugs and chemicals were purchased commercially and were of analytical grade.

Experimental Design

Induction of experimental hepatotoxicity

Hepatotoxicity was induced by injecting CCl_4 intraperitonially at a dose of 1 ml/kg body weight for 7 consecutive days.

Evaluation of hepatoprotecive activity

Animals were divided into five groups, consisting of six animals each. The rat dose was calculated on the basis of the surface area ratio.[5]

Group I	Control (Normal saline 10 ml/kg, *p.o*)
Group II	CCl_4 (1 ml/kg, *i.p*)
GroupIII	Silymarin (25 mg/kg, *p.o*)+CCl_4 (1 ml/kg, *i.p*)
GroupIV	LIV-first (0.5ml/kg, *p.o*)+CCl_4 (1 ml/kg, *i.p*)
GroupV	LIV-first (1ml/kg, *p.o*)+CCl_4 (1 ml/kg, *i.p*)

All the groups were treated for 7 consecutive days.[6] At the end of this period, animals were kept overnight fasting and were sacrificed. Blood samples were withdrawn, serum separated and estimated for biochemical parameters. Liver tissues were removed for the determination of antioxidant enzyme levels and histopathological examinations.

Measurement of Biochemical Parameters

Blood samples were collected from retro-orbital plexus under ether anaesthesia and the serum was used for the assay of marker enzymes namely SGPT, SGOT, ALP and bilirubin. The enzyme levels were assayed using standard kits obtained from Agappe Diagnostics Pvt. Ltd, Kerala.[7-10]

The liver homogenate was prepared and the clear supernatant was used for the estimation of lipid peroxidation (MDA),[11,12] total protein,[13] reduced glutathione (GSH)[14,15] and antioxidant enzymes viz. catalase (CAT)[14,16] and superoxide dismutase (SOD)[17,18] levels.

Histopathological Examination

A portion of liver tissue from each group was preserved in a 10% formaldehyde solution for histopathological studies. Haematoxylin and eosin were used for staining and later the microscopic slides of the liver cells were photographed at a magnification of ×100.

Statistical Analysis

Values were represented as mean±SEM. Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test using statistical package for social sciences (SPSS) version 10.0. $P<0.05$ was considered significant.[19]

RESULTS

Biochemical Parameters

The animals treated with CCl_4 exhibited a significant ($P<0.01$) rise in SGOT, SGPT, ALP and bilirubin levels when compared to the control group. This was significantly ($P<0.01$) reduced after treatment with LIV-first, which was almost similar to that of silymarin [Table 1].

Lipid Peroxidation

The liver MDA, which is an index of tissue lipid peroxidation, was found to be significantly ($P<0.01$) higher in the CCl_4-treated group than measured in the control group. Treatment with LIV-first decreased the elevated MDA levels. The MDA level for silymarin was also found to be significantly decreased.

Total Protein

Total protein level was significantly ($P<0.01$) reduced in the CCl_4-treated group when compared to the control and was significantly elevated in the LIV-first-treated groups. This was comparable to that of silymarin-treated group.

Antioxidant Enzymes and Glutathione Levels

The levels of antioxidant enzymes such as CAT and SOD and GSH were decreased significantly ($P<0.05$) after CCl_4 treatment and was significantly ($P<0.01$) elevated in LIV-first-treated group. This was comparable with that of silymarin-treated group [Table 2].

Histopathology

The histopathological examination showed that treatment with CCl_4 caused typical centribular hepatocytic steatosis (both macrovesicular and microvesicular) and necrosis, limiting plate necrosis, apoptosis, especially in the periportal hepatocytes and portal triaditis as compared with control liver. Liver tissues exposed to LIV-first and silymarin were almost similar to the control in histology, size and

Table 1: Effect of LIV-first on serum biochemical parameters against CCl_4-induced hepatotoxicity in rats

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Bilirubin (mg/dl)
Control (10 ml/kg saline, *p.o*)	32.6±2.83	26.02±9.27	16.77±4.14	0.46±0.02
CCl_4 (1 ml/kg, *i,p*)	95.26±3.80[a]	75.46±7.95[a]	47.39±6.61	1.07±0.007[a]
LIV-first (0.5 ml/kg, *p.o*+CCl_4	58.26±3.8[b]	48.46±1.25[b]	27.39±2.5[a]	0.83±0.018[b]
LIV-first (1 ml/kg, *p.o*)+CCl_4	35.13±4.34[b]	36.46±3.29[b]	17.43±3.35[b]	0.67±0.002[b]
Silymarin (25 mg/kg, *p.o*)+CCl_4	25.14±8.82[b]	18.45±1.01[b]	10.94±4.02[b]	0.41±0.004[b]

Values are expressed as mean±SEM; n=6 in each group; [a]$P<0.01$ vs control group; [b]$P<0.01$ vs CCl_4-treated group (ANOVA followed by Dunnett's test)

staining properties and showed only mild congestion. In the formulation-treated group, there was reduction in inflammation and it significantly prevented the degeneration of hepatocytes. Thus, histological examination clearly demonstrated the protection of liver against CCl_4 cytotoxicity.

DISCUSSION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine.[20]

In the assessment of liver damage by CCl_4, the determination of enzyme levels was largely used. Serum SGPT, SGOT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. In this study, an increase in the activities of SGPT, SGOT, ALP and bilirubin in serum evidenced the CCl_4-induced hepatocellular damage.[21-24] The reduction of CCl_4-induced elevated plasma activities of these enzyme levels in animals treated with the formulation showed their ability to restore the normal functional status of the damaged liver.[23,24]

The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation.[22] The result suggests that there was a dramatic increase in lipid peroxidation after CCl_4 treatment and it was inhibited by the treatment with the formulation revealing that it exhibits potent hepatoprotective activity.

Measurement of protein concentration was mainly used to calculate the level of purity of a specific protein. High doses of CCl_4 cause depletion of total proteins indicating tissue damage which was also evidenced in this study. Treatment with CCl_4 significantly depleted GSH, CAT and SOD stores indicating that they were used for the detoxification of toxic metabolites of the drug. The formulation restored the antioxidant enzyme levels significantly and reduced the CCl_4-induced oxidative injury, thus proving its antioxidant potential.[13]

The histopathological examination of the liver of the control group showed normal hepatocytes with portal triad [Figure 1]. The liver section of CCl_4-treated rats showed typical centribular hepatocytic steatosis (both macrovesicular and microvesicular) and necrosis, limiting plate necrosis, apoptosis especially in the periportal hepatocytes and portal triaditis [Figure 2]. This could be due to the formation of highly reactive free radicals

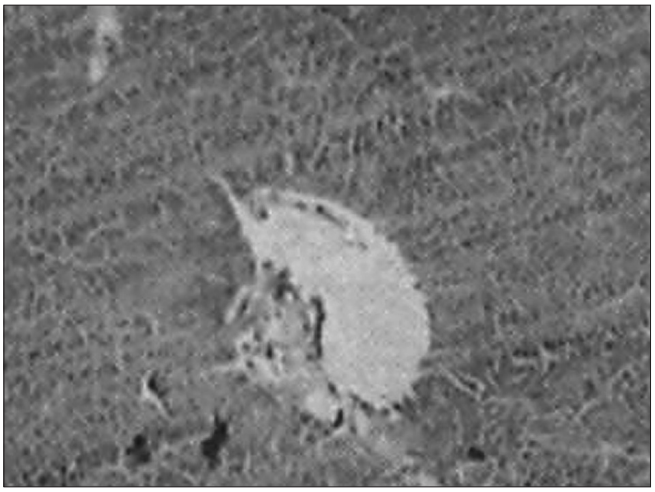

Figure 1: Photomicrograph of rat liver showing normal hepatocytes and central portal vein (H and E, ×100)

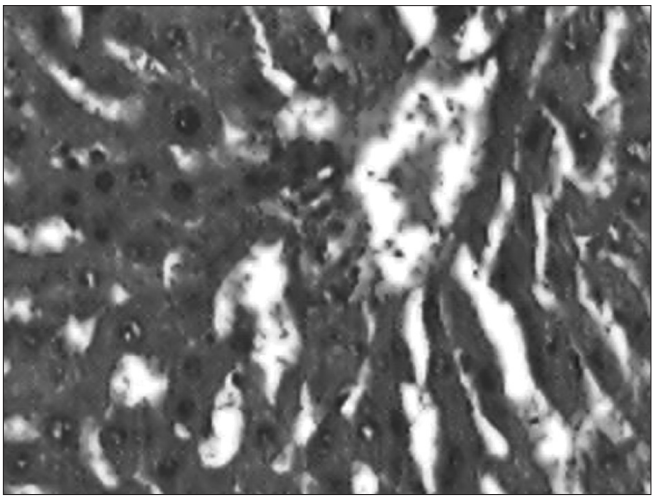

Figure 2: Photomicrograph of rat liver treated with CCl_4 showing necrosis, centrilobular steatosis and portal triaditis (H and E, ×100)

Table 2: Effect of LIV-first on liver malondialdehyde, total protein, glutathione and antioxidant enzymes against CCl_4-induced hepatotoxicity in rats

Treatment	MDA (μmol/g tissue)	Protein (μg/ml)	GSH (μmoles of GSH/g wet tissue)	CAT (units/mg liver protein)	SOD (units/mg liver protein)
Control (10 ml/kg saline, *p.o*)	26.4±0.41	23.01±0.16	42.71±0.99	57.27±1.62	89.46±1.04
CCl_4 (1 ml/kg, *p.o*)	50.27±0.61[a]	8.21±0.62[a]	15.83±2.11[b]	25.80±1.61[a]	36.18±2.73[a]
LIV-First (0.5 ml/kg, *p.o*)	35.29±0.50[c]	14.09±0.57[c]	20.83±0.71[c]	36.91±1.07[c]	71.72±1.24[c]
LIV-First (1 ml/kg, *p.o*)	32.62±0.50[c]	17.75±0.23[c]	30.00±0.36[c]	46.78±0.22[c]	82.14±0.37[c]
Silymarin (25 mg/kg, *p.o*)	30.41±0.22[c]	19.7±0.18[c]	33.85±1.52[c]	46.28±0.51[c]	78.92±4.70[c]

Values are expressed as mean±SEM; n=6 in each group; [a]$P<0.01$ and [b]$P<0.05$ vs control; [c]$P<0.01$ vs CCl_4

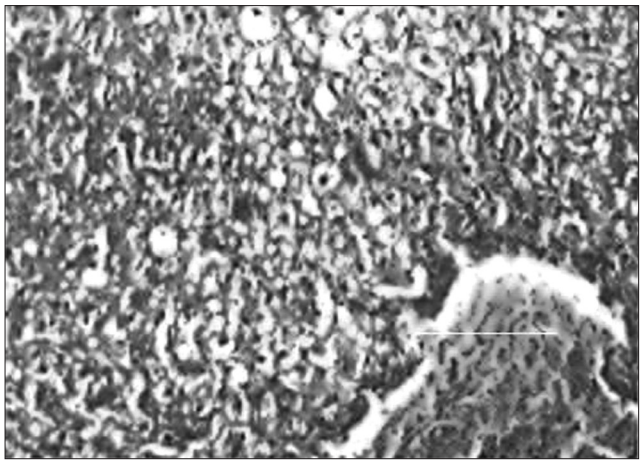

Figure 3: Photomicrograph of rat liver treated with CCl_4+Liv-First (0.5 ml/kg) showing centrilobular venous congestion and diffuse macrovesicular steatosis (H and E, ×100)

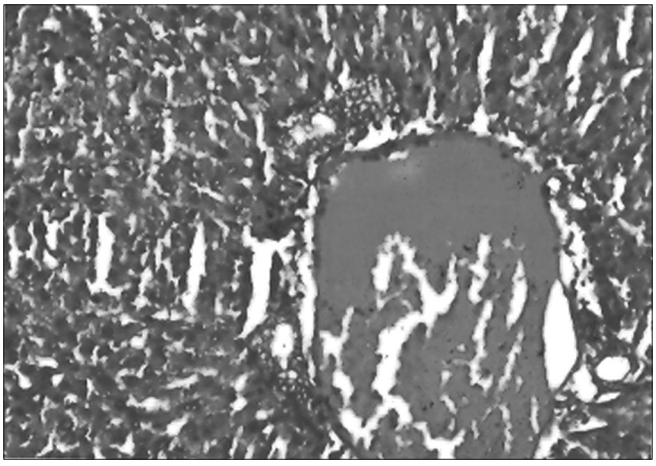

Figure 4: Photomicrograph of rat liver treated with CCl_4+Liv-First (1 ml/kg) showing centrilobular venous congestion and diffuse macrovesicular steatosis (H and E, ×100)

because of oxidative stress caused by CCl_4. Simultaneous administration of formulation along with CCl_4 prevented these effects [Figures 3 and 4]. Thus, histopathological studies revealed that concurrent administration of CCl_4 with the formulation exhibited protection of liver cells, which further confirmed the above results.

CONCLUSION

The results of this study clearly demonstrated that the formulation exhibited potent hepatoprotective activity against CCl_4-induced hepatic damage in rats. This may be due to their antioxidat and free radical scavenging properties. Further studies are needed to isolate and purify the active principles involved in the individual plants of the formulation for confirming the hepatoprotective efficacy.

REFERENCES

1. Guillouzo A. Hepatotoxicity. In: Frazier JM, editor. *In vitro* toxicity test applications to safety evaluation. New York: Marcel Dekker, Inc. p. 45-53.
2. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. 3rd ed. London: Oxford University Press; 1999.
3. Kim J. An overview of drug-induced liver disease. US Pharm 2005;11:HS-10-HS-21.
4. Clawson GA. Mechanisms of carbon tertrachloride hepatotoxicity. Pathol Immunopathol Res 1989;8:104-12.
5. Ghosh MN. Fundamentals of experimental pharmacology. 3rd ed. Kolkata: Hilton and Co; 2005. p. 192.
6. Samudram P, Rajeshwari H, Vasuki R, Geetha A, Sathiamoorthy P. Hepatoprotective activity of Bi-herbal ethanolic extract on CCl_4 induced hepatic damage in rats. Afr J Biochem Res 2008;2:61-5.
7. GPT/ALAT UV Kinetic Test [package insert]. Ernakulam (KL): Agappe Diagnostics Pvt. Ltd; 2006.
8. GOT/ASAT UV Kinetic Test [package insert]. Ernakulam (KL): Agappe Diagnostics Pvt. Ltd; 2006.
9. Alkaline Phosphatase P-NPP Method [package insert]. Ernakulam (KL): Agappe Diagnostics Pvt. Ltd; 2006.
10. Bilirubin Total and Direct [package insert]. Ernakulam (KL): Agappe Diagnostics Pvt. Ltd; 2006.
11. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochem 1979;95:351-8.
12. Naik RS, Mujumdar AM, Ghaskadbi S. Protection of liver cells from ethanol toxicity by curcumin in liver slice culture *in vitro*. J Ethnopharmacol 2004;95:31-7.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol reagent. J Biol Chem 1951;193:265-75.
14. Pari L, Latha M. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipd peroxidation in STZ diabetic male *Wistar* rats. BMC Compliment Altern Med 2004;4:6882-4.
15. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
16. Sinha AK. Colorimetric assay of catalase. Analytical Biochem 1972;47:389-94.
17. Budhwar R, Kumar S. Prevention of chromate induced oxidative stress by alpha-lipoic acid. Indian J Exp Biol 2005;43:531-5.
18. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170-5.
19. Gennaro AR. Remigton: The science and practice of pharmacy. 19th ed. Vol.1. Easton PA: MacPublishing Co; 1995. p. 111.
20. Malhotra S, Singh AP. Hepatoprotective natural products. Available from: http://www.siv.edu/ebl/leaflets/hepato.htm. [cited on 2006 Nov 11].
21. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol 1954;7:322-6.
22. Drotman RB, Lawhorn GT. Serum enzymes as indicators of chemically induced liver damage. Drug Chem Toxicol 1978;1:163-71.
23. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.
24. Pari L, Amali DR. Protective role of tetrahydrocurcumin, an active principle of turmeric on chloroquine-induced hepatotoxicity in rats. J Pharm Pharmaceut Sci 2005;8:115-23.

Source of Support: Nil, **Conflict of Interest:** None declared.