

In vitro antioxidant and *in vivo* prophylactic effects of heptyl 3-(2-formylphenyl) propanoate isolated from *Delonix elata* L. against hepatotoxicity in CCl₄ intoxicated rats

Pradeepa Krishnappa^{1,2}, Krishna Venkatarangaiah^{2,*}, Venkatesh^{2,3} and Santosh Kumar Shimoga Rajanna²

¹Department of Biotechnology, M.S. Ramaiah Institute of Technology, Bengaluru-560054, Karnataka, India

²Department of Post Graduate Studies and Research in Biotechnology, Kuvempu University, Shankaraghatta-577 451, Karnataka, India

³Department of Biochemistry, Indian Institute of Science, Bengaluru-560012, Karnataka, India

Received 13 July 2015; Revised 08 December 2016

Delonix elata L. (Family Caesalpiniaceae) has been used in the folklore medicines in Karnataka, India to cure liver and rheumatic problems. Bioassay guided fractionation of the chloroform leaf extract of *D. elata* has resulted in the isolation of heptyl 3-(2-formylphenyl) propanoate (HFP). The prophylactic activity of leaf chloroform extract (DLC) and the isolated constituent were evaluated against CCl₄-induced hepatotoxicity in rats. The treatment with DLC and HFP with concomitant CCl₄ intraperitoneal injection significantly reduced the elevated plasma levels of AST, ALT, ALP, triglycerides, and cholesterol compared with the CCl₄ injected group. DLC and HFP showed significant prophylactic effect on activities of SOD, CAT, GPx, GST, and also significantly inhibited the elevated levels of MDA in liver homogenates. Results obtained in biochemical assays were well supported by histological observations. The results revealed that the hepatoprotective activity of DLC and HFP is significant and on par with the standard drug silymarin. To clarify the influence of the DLC and HFP on the protection of oxidative-hepatic damage, *in vitro* antioxidant properties were examined. Free radical scavenging activity was exhibited by both, the extract and the constituent. The results suggest that the DLC and HFP could protect the hepatocytes from CCl₄-induced liver damage perhaps, by their anti-oxidative effect on hepatocytes.

Keywords: Antioxidant activity, *Delonix elata*, Hepatoprotective activity, Heptyl 3-(2-formylphenyl) propanoate.

IPC code; Int. cl. (2015.01)– A61K 36/00, A61K 36/48, A61K 39/00, A61P 1/16

Introduction

Liver is the largest and an essential organ of human body, which regulates several important metabolic functions. It detoxifies the exogenous xenobiotics and drugs. While performing several detoxifications, liver undergoes stress, leading to liver diseases ending in liver damage and serious health problems and death. In liver diseases, excessive oxidative stress contributes to the progression and pathological findings of disease and serves as a prognostic indicator. Hepatocellular carcinoma (HCC) is one of the most frequent tumor types worldwide. It is the fifth most common cancer and the third leading cause of cancer death¹. Long term hepatitis B virus (HBV) infection is a major risk factor in pathogenesis of chronic liver diseases, including hepatocellular

carcinoma (HCC). HBV and HCC are associated with oxidative stress, which can damage cellular molecules like lipids, proteins, and DNA during chronic infection¹. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants has been proposed as therapeutic agents, as well as drug co-adjuvants, to counteract liver damage. In spite of tremendous advancement in the modern medicine, hepatoprotective drugs are limited and are associated with severe side-effects². The use of natural remedies for the treatment of liver diseases has a long history, starting with the *Ayurvedic* treatment, and extending to the Chinese, European and other systems of traditional medicines³. There has been widespread recognition that indigenous drugs used traditionally by ethnic tribes or societies across the globe can provide respite to patients with hepatic disorders.

Delonix elata L. is a deciduous tree belonging to the family Caesalpiniaceae and is sparsely distributed in the dry forests of India and also found in African

*Correspondent author

Tel: (+91) 08282-256235

Fax: (+91) 08282-256255

E-mail: krishnabiotech2003@gmail.com

Supplementary figures are available online only.

countries. Traditional medical practitioners residing in the villages of Chitradurga District, Karnataka, India have been using the leaf extract to cure jaundice, bronchial, and rheumatic problems. In our previous study, stem bark extract of *D. elata* has shown potential antioxidant and hepatoprotective activity against carbon tetrachloride (CCl₄)-induced liver toxicity in rats⁴. *In vitro* antioxidant activity of the leaves extract of *D. elata* has been reported earlier⁵. However, detailed investigation on phytochemical analysis to isolate bioactive constituent and hepatoprotective activity of *D. elata* leaves extract is not yet reported. Thus, the present investigation was undertaken to isolate bioactive phytoconstituent from *D. elata* leaf extract and evaluation of hepatoprotective activity against CCl₄-induced liver damage in rats.

Materials and Methods

Chemicals

Silymarin was purchased from Micro labs Bangalore, India; carbon tetrachloride, petroleum ether, chloroform, ethanol, butylated hydroxyl anisole (BHA), acrylamide, N,N-methylene bisacrylamide, sulfanilamide, sodium nitroprusside (SNP), trichloroacetic acid (TCA), folin-ciocalteu reagent were purchased from Merck Ltd, Mumbai, India; quercetin, thiobarbituric acid (TBA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), nicotinamide adenine dinucleotide (NADH), ferrozine, nitroblue tetrazolium disodium salt (NBT), phenazine methosulfate (PMS) and O-dianisidine were obtained from Sigma-Aldrich, St. Louis, USA; gallic acid, ascorbic acid, ferrous chloride and potassium persulfate were procured from Himedia, India. All the chemicals used were of analytical grade. Water was purified using Milli-Q system from Millipore, Bedford, USA. Diagnostic test kits for the assay of liver function markers were purchased from Robonik India Pvt Ltd, Mumbai, India.

Preparation of plant extracts

The leaves of *D. elata* were collected from Chitradurga, Karnataka, India in October 2012. The plant material was identified and authenticated by Dr Manjunatha BK, by matching with the voucher specimen deposited at the Kuvempu University Herbarium specimen⁶. The powdered material (500 g) was defatted in petroleum ether using soxhlet extraction. Further, hot extraction was carried out with defatted material with chloroform (1.5 L, 50–70 °C, Merck, Mumbai, India) to obtain crude

extract and named it as *D. elata* chloroform extract (DLC) (Yield: 3.4 %).

Isolation of bioactive compound

DLC was subjected for TLC studies using the solvent system hexane: chloroform (9:1), which showed separation of distinct spot, with R_f value, 0.67. About 20 g of crude extract was subjected to silica gel column chromatography (60×4 cm, 60–120 mesh, 200 g), eluted by gradient elution method using hexane/chloroform in combination. Initially, column was run with pure hexane (100 mL) followed by hexane/chloroform in 9:1 combination (130 mL). A total of 23 fractions (10 mL each) were eluted and fractions 12 to 17 yielded 347 mg pure compound. The purity of the isolated compound was monitored by TLC examination. Melting point of the isolated compound was determined using scientific melting point apparatus. Characterization of the isolated compound was carried out by IR, ¹H-NMR, ¹³C-NMR, and MS studies.

Acute toxicity study

An acute toxicity study was conducted for DLC and its constituent by the Up-and-Down procedure⁷. DMSO (1 % v/v) was used as a vehicle to suspend the drugs and administered orally. Animals were observed individually for first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 days for changes in their behavioural pattern and mortality.

CCl₄-induced hepatotoxicity

Wistar albino rats of either sex, weighing 180–200 g were used for the study. Animals were housed at 25±1 °C, humidity 55–60 % in the Department of Biotechnology, Kuvempu University, Karnataka, India. They were fed with standard commercial pellet diet (Sai Durga feeds and foods, Bangalore) and water *ad libitum* during the experiment. The study was approved by the Institutional Ethical Committee (NCP/IAEC/CL/13/12/2010-11).

Rats were divided into five groups consisting of six animals in each group. Group 1 served as vehicle control and received 1% (v/v) DMSO (1 mL/kg body weight (b.w.), p.o); group 2 (Toxic control) received 50 % CCl₄ in olive oil (1 mL/kg b.w., i.p); group 3 received DLC (300 mg/kg b.w., p.o); group-4 received isolated constituent (60 mg/kg b.w., p.o); group-5 received standard drug silymarin (25 mg/kg b.w., p.o) once in a day. Treatment duration was 15 days and all the groups received intraperitoneal dose of 50 % CCl₄ after every 72 h⁸. At the end of the

experimental period, animals were sacrificed and blood was collected. The liver tissue was excised and used for biochemical assays.

Measurement of serum biochemical parameters

AST, ALT, ALP, serum total cholesterol, total bilirubin, total protein and triglyceride levels in serum from each animal were determined using biochemical analyzer in conjugation with commercial assay kits (both of Robonik India Pvt Ltd, New Mumbai).

Assessment of antioxidant enzyme activities

Liver tissue (10 %) was homogenized in ice cold normal saline and centrifuged at 4000 rpm for 5 min. The supernatant was used for the following assays. The activities of superoxide dismutase⁹, catalase¹⁰, glutathione peroxidase¹¹, and glutathione-S-transferase¹² were determined.

Determination of lipid peroxidation

MDA, which is one of lipid peroxidation product, was determined¹³ by the method of Ohkawa *et al.* In brief, 0.5 mL of the 10 % homogenate was mixed with 100 μ L of 0.2 mM FeCl₃, 2 mL reaction mixture (0.25 N HCl containing 15 % TCA, 0.30 % TBA and 0.05 % BHA). Heated at 80 °C for 1 h, cooled, and then centrifuged at 1,500 rpm. The supernatant was collected. Lipid peroxidation products were estimated by measuring the concentration of thiobarbituric acid reaction substances (TBARS) in fluorescence at 530 nm.

Histopathology of liver

Liver tissues were fixed in 10 % formalin–saline for 18 h, embedded in paraffin. Sections were prepared by using a rotary microtome, and stained with haematoxylin–eosin dye, mounted in DPX with a cover slip and histological changes were observed under microscope¹⁴.

Determination of *in vitro* antioxidant activity

The free radical-scavenging activity was measured using DPPH¹⁵ according to the procedure described by Braca *et al.* The ability of leaves extracts to scavenge hydroxyl radical was determined¹⁶ by the method of Klein *et al.* Superoxide anion radical scavenging activity was measured¹⁷ according to the method of Nishimiki *et al.* Nitric oxide radical scavenging assay was determined¹⁸ using the method of Marcocci *et al.* Metal chelating activity of leaves extracts was measured¹⁹ by adapting the method of Dinis *et al.* The method of Halliwell and Gutteridge, was used to determine the lipid peroxidation

inhibition assay²⁰. The antioxidant activity of BHT, ascorbic acid, EDTA, and curcumin was determined for comparison. The IC₅₀ (the concentration required to scavenge 50 % of radical) value was calculated using the formula:

$$IC_{50} = [(\Sigma C / \Sigma I) \times 50]$$

where ΣC is the sum of extracts concentrations used to test and ΣI is the sum of percentage of inhibition at different concentrations.

Statistical analysis

Results are presented as mean \pm standard errors (SE). Duncan's test was used to evaluate the significance of difference between groups. The differences in values at $p < 0.05$ or $p < 0.01$ were regarded as statistically significant. GraphPad Prism 5 software was used for statistical analysis.

Results and Discussion

Isolation of bioactive compound

Melting point of the compound was 72 °C. The IR spectrum exhibits characteristic absorption bands at 3432 cm^{-1} for OH group and 1736 and 1716 for two carbonyl groups, and at 1173 cm^{-1} for C-O group (Supplementary Fig. S1). The ¹³C NMR spectrum (Supplementary Fig. S2) indicates the presence of a keto or aldehyde carbonyl group by exhibiting a signal at δ 203.06. The presence of an aromatic ring system by exhibiting signals at δ 144.78, 129.01, 128.20, 127.47, and 113.70. The signals at δ 14.09, 22.07, 22.67, 25.01, 25.92, 26.38, 28.68, 31.91, and 34.41 suggest the presence of a long chain hydrocarbon. The signals at δ 64.41, 50.84, and 43.90 suggest the presence of a hetero atom (O or N) in the molecule. The ¹H NMR spectrum (Supplementary Fig. S3) suggests that the signal at δ 9.76 may be due to an aldehyde proton. The doublet signal at δ 7.54 and a triplet at 6.98 suggest the presence of aromatic protons. The signals at δ 4.75 and 4.38 suggest the presence of protons of oxygen function. The signals at δ 0.89 and between 1.20 and 2.47 suggest the presence of a long chain hydrocarbon. ESI MS: $m/z = 276$ [M+H]⁺ (Supplementary Fig. S4). Based on the spectral details, the isolated compound is characterized as Heptyl 3-(2-formylphenyl) propanoate (Fig. 1) and it is abbreviated as HFP.

Acute toxic studies

The LD₅₀ of the DLC and HFP was found to be 3000 and 600 mg/kg b.w., respectively. One tenth of

the dose i.e., 300 and 60 mg/kg b.w. was considered as safer dose for oral administration.

Prophylactic effect of the extract against CCl₄-induced liver damage

CCl₄-induced hepatotoxicity model is being widely used in studies on therapies against various hepatic diseases, because CCl₄-induced liver damage shares similar mechanism with viral hepatitis²¹, drug/chemicals-induced hepatopathy, and oxidative stress^{22,23}. CCl₄ is metabolized to the trichloromethyl radical (•CCl₃) and proxy trichloromethyl radical (•OCCl₃) by cytochrome P450 2E1 enzyme²⁴. These radicals bind covalently to the macromolecules and cause peroxidative degradation of cellular lipid membrane that causes the loss of integrity of cell membranes, and the necrosis of hepatocytes²⁵. Liver damage can be assessed by biochemical studies. AST, ALP, and ALT levels are most frequently used in the diagnosis and management of liver diseases. AST, ALP, and ALT were present in high concentration in hepatocytes, while they leaked into the circulation when hepatocytes or their membranes were damaged²⁶. In the present study, this process is evidenced by an elevation in the serum markers viz., AST (561.97±9.64 U/L), ALT (161.90±2.37 U/L), ALP (400.10±4.91 U/L), total bilirubin (14.07±1.04 mg/dL), triglyceride (215.97±5.48 mg/dL), total cholesterol (274.00±3.51 mg/dL) after CCl₄ administration in rats. CCl₄ also induces cellular hypomethylation, leading to inhibition of protein synthesis (possibly through ribosomal RNA

hypomethylation) and defects in lipid and protein metabolism²². In the present study, significant decrease in total proteins level (5.84±0.09 g/dL) was observed as compared to the control group (1 % DMSO). On the contrary, the increased levels of these liver function markers were brought down nearer to normalcy due to the amelioration effect of the DLC and HFP (Table 1), particularly the group treated with HFP showed a significant decrease in total bilirubin, AST, ALT, total cholesterol, and triglyceride ($p < 0.01$) along with a significant recovery in total proteins level ($p < 0.01$).

MDA level in liver tissue is used to reflect the extent of lipid peroxidation in hepatocytes, as MDA is one of the end-products of polyunsaturated fatty acid peroxidation²⁷. In the present study, a 2 fold increase in levels of MDA was noticed in CCl₄ intoxicated rat liver (1.11±0.01 nmol/mg of protein) as compared with the liver homogenates of control group animals with MDA level of 0.64±0.01 nmol/mg of protein. These by-products can form protein as well as DNA adducts and may contribute to hepatotoxicity²². Whereas the HFP administered animals significantly reduced the levels of MDA towards normalcy (0.74±0.01 nmol/mg of protein, $p < 0.01$) (Table 2).

SOD, CAT, GPx, and GST are the major antioxidant enzymes responsible for the defense against potentially free radicals that cause oxidative stress. CCl₄ not only initiates lipid peroxidation but also reduces tissue SOD, CAT, GPx, and GST activities and this depletion may result from oxidative modification of these proteins²⁸. Antioxidant enzymes estimation in the liver homogenates of CCl₄ intoxicated rats revealed significant decrease in the activity of SOD, CAT, GPx, and GST compared to the control group (Table 2). DLC and HFP administration effectively protected against the loss of these antioxidant activities after CCl₄ administration.

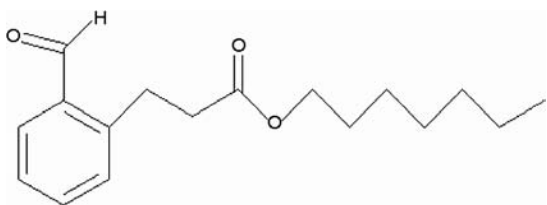


Fig. 1—Structure of Heptyl 3-(2-formylphenyl) propanoate

Table 1—Prophylactic effect of DLC and HFP on CCl₄-induced liver damage

Groups	ALP (U/L)	AST (U/L)	ALT (U/L)	Total bilirubin (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (g/dL)
Control (1 % DMSO)	223.77±5.85	201.20±3.84	61.75±3.34	0.04±0.01	150.00±2.00	173.50±2.75	8.28±0.15
CCl ₄ (50 %) (1 mL/kg)	400.10±4.91	561.97±9.64	161.90±2.37	14.07±1.04	274.00±3.51	215.97±5.48	5.84±0.09
DLC (300 mg/kg)	294.87±8.12**	339.53±4.62**	101.20±3.38**	0.38±0.01**	201.1±2.00**	186.10±1.38**	7.12±0.06**
HFP (60 mg/kg)	311.37±1.47**	306.56±2.0**	87.81±3.5**	0.21±0.01**	221±1.03**	181.21±2.31**	7.02±0.05*
Silymarin (25 mg/kg)	241.87±1.88**	316.13±5.98**	84.63±2.26**	0.10±0.01**	186.00±3.06**	162.23±1.12**	7.86±0.16**

Each value represents mean±S.E. of 6 animals. Symbols represent statistical significance. * $p < 0.05$, ** $p < 0.01$, as compared to CCl₄ intoxicated group.

Table 2—Effects of chloroform extract of *D. elata* leaves extract and its constituent on hepatic antioxidant enzymes activities

Groups	GPx (nmol NADPH/min/mg of protein)	GST (nmol/min/mg of protein)	CAT (nmol/min/mg of protein)	SOD (U/mg of protein)	MDA (nmol/ mg of protein)
Control (1 % DMSO)	163.72±0.19	422.7±1.9	484.82±3.17	12.47±0.38	0.64±0.01
CCl ₄ (50 %) (1 mL/kg)	87.14±0.35	209.58±1.0	214.6±0.7	5.16±0.07	1.11±0.01
DLC (300 mg/kg)	127.82±0.14**	335.4±0.4**	348.5±1.19**	7.25±0.05**	0.89±0.01**
HFP (60 mg/kg)	104.47±0.22**	340.58±0.37**	308.80±2.12**	8.63±0.1**	0.74±0.01**
Silymarin (25 mg/kg)	140.14±1.11**	362.2±1.0**	362.85±0.05**	8.73±0.14**	0.64±0.01**

Each value represents mean±S.E. of 6 animals. Symbols represent statistical significance. * $p < 0.05$, ** $p < 0.01$, as compared to CCl₄ intoxicated group.

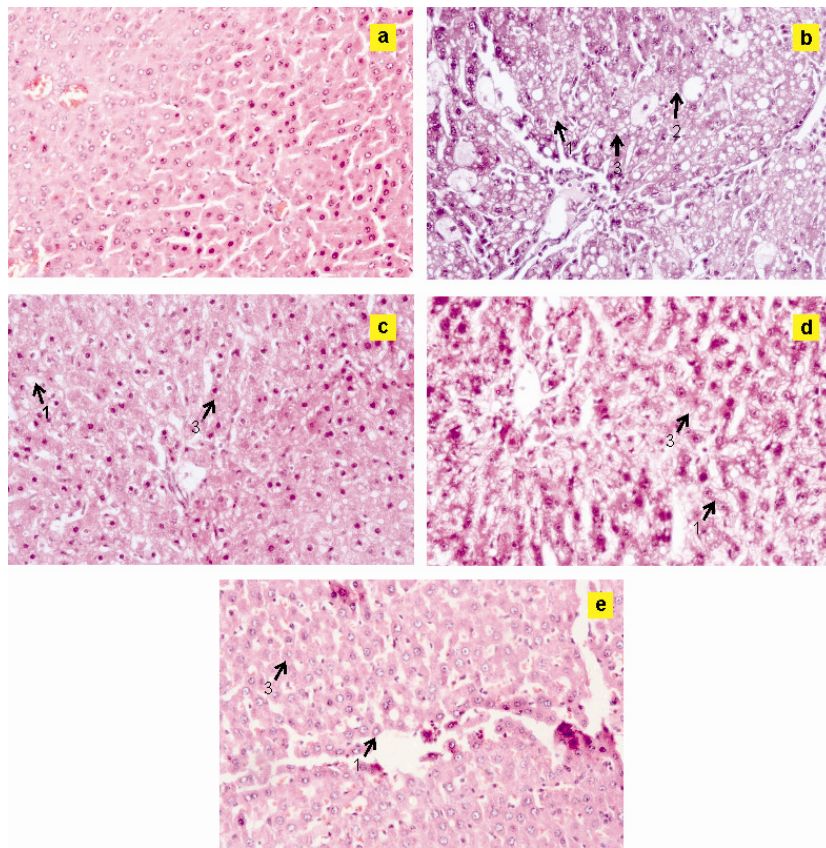


Plate 1—Histopathology of liver tissue: a) Normal control, b) CCl₄ treated, c) DLC pretreated + CCl₄, d) HFP pretreated + CCl₄, and e) silymarin pretreated + CCl₄

HFP showed significant prophylactic effect on activities of SOD (8.63±0.1 U/mg of protein, $p < 0.01$), CAT (308.80±2.12 nmol/min/mg of protein, $p < 0.01$), GPx (104.47±0.22 nmol NADPH/min/mg of protein, $p < 0.01$), and GST (340.58±0.37 nmol/min/mg of protein, $p < 0.01$).

Histopathological examination of liver tissue

Histopathological observations of liver sections from the control group have shown normal cellular architecture with distinct hepatic cells, sinusoidal

spaces, a central vein, and compact arrangement of hepatocytes without fatty lobulation (Plate 1a). In contrast, the CCl₄ intoxicated group revealed the most severe damage in the liver showed massive fatty changes, necrosis, ballooning degeneration, broad infiltration of lymphocytes, and the loss of cellular boundaries²⁴ (Plate 1b). Animals administered with DLC exhibited liver protection against CCl₄-induced liver damage, as evident by the presence of hepatic cords with moderate fatty change, necrosis and few inflammatory cells (Plate 1c). The liver sections of the

Table 3—*In vitro* antioxidant activity of chloroform extract of *D. elata* leaves and its constituent

S. No.	Activity	DLC concentration (μg)	% of inhibition DLC	HFP concentration (μg)	% of inhibition HFP
1	DPPH radical scavenging activity	50	20.75 \pm 0.27	25	11.40 \pm 0.57
		100	43.95 \pm 0.17	50	19.19 \pm 0.04
		150	59.05 \pm 0.28	100	34.8 \pm 0.66
2	Superoxide radical scavenging activity	100	28.65 \pm 0.25	25	18.12 \pm 0.35
		200	54.16 \pm 0.07	50	31.11 \pm 0.11
		300	71.57 \pm 0.20	100	51.12 \pm 0.28
3	Hydroxyl radical scavenging assay	100	21.38 \pm 0.31	25	5.92 \pm 0.28
		250	36.55 \pm 0.18	50	9.64 \pm 0.11
		500	45.25 \pm 0.25	100	14.13 \pm 0.33
4	Nitric oxide radical scavenging activity	100	13.48 \pm 0.02	25	13.48 \pm 0.02
		250	25.42 \pm 0.15	50	27.47 \pm 0.22
		500	47.04 \pm 0.19	100	47.01 \pm 0.09
		1000	13.48 \pm 0.02	25	7.68 \pm 0.42
5	Metal chelating	2000	25.42 \pm 0.15	50	13.61 \pm 0.21
		3000	47.04 \pm 0.19	100	20.42 \pm 0.56
		500	15.16 \pm 0.18	25	3.58 \pm 0.13
6	Lipid peroxidation inhibition	1000	25.52 \pm 0.47	50	5.32 \pm 0.42
		1500	43.50 \pm 0.02	100	9.63 \pm 0.08

The results shown are averages of three independent experiments, values are mean \pm SE.

Table 4—IC₅₀ values of DLC and HFP for *in vitro* antioxidant activity

S. No.	Activity	DLC (μg)	HFP (μg)	Standard (μg)
1	DPPH radical scavenging	121.2 \pm 0.17	133.81 \pm 2.1	85.26 \pm 0.41 (BHT)
2	Superoxide radical scavenging	194.32 \pm 0.47	87.19 \pm 0.8	101.13 \pm 1.0 (Ascorbic acid)
3	Hydroxyl radical scavenging	969.13 \pm 1.17	294.71 \pm 1.2	125.66 \pm 1.2 (BHT)
4	Nitric oxide radical scavenging	494.56 \pm 1.81	99.47 \pm 5.2	98.34 \pm 0.74 (Curcumin)
5	Metal chelating assay	581.75 \pm 4.78	209.78 \pm 7.5	31.52 \pm 0.0 (EDTA)
6	Lipid peroxidation inhibition	3564.03 \pm 11.22	472.20 \pm 14.2	—

The results shown are averages of three independent experiments, values are mean \pm SE

rats pretreated with HFP (Plate 1d) showed more or less normal lobular pattern with a mild degree of fatty change, necrosis, and lymphocyte infiltration almost comparable to the silymarin treated group (Plate 1e).

Determination of *in vitro* antioxidant activity

In order to clarify the mode of action of extract and its constituent, *in vitro* antioxidant study was carried out. The radical scavenging potentialities of the DLC and HFP tested at three different concentrations are given in the Table 3-4.

In vivo mechanism of action against oxidative stress is very complex process and a single *in vitro* chemical method is not sufficient to validate the antioxidant properties. Therefore, different assays like reducing power assay, radicals scavenging assays, metal chelating and lipid peroxidation inhibition activities have been carried out to evaluate various mechanisms such as, prevention of chain initiation, binding with transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reducing capacity, and radical scavenging ability of the extracts towards ROS/RNS.

In the present investigation, HFP exhibited a significant dose-dependent scavenging effects on superoxide anion radical, nitric oxide radical, hydroxyl radical, and DPPH radical. The effect of DLC and HFP on metal chelating, lipid peroxidation inhibition, and reducing power are also given in Table 3-4. According to the results obtained, chloroform extract and its constituents inhibited lipid peroxidation in liver homogenate.

Conclusion

Results revealed that the chloroform leaf extract and the isolated constituent, HFP showed consistent and concentration dependent antioxidant and hepatoprotective activities against CCl₄-induced liver injury. These results support a beneficial relationship between the antioxidant activity and hepatoprotective effect of leaf extract of *D. elata*.

Acknowledgement

The authors are grateful to the Principal, National college of Pharmacy, Shimoga, Karnataka for the support. The work was partially supported by Department of Biotechnology, New Delhi (BT/PR11505/SPD/24/337/2008 & BT/PR9128/INF/22/190/2013).

References

- Ha H L, Shin H J, Feitelson M A and Yu D Y, Oxidative stress and antioxidants in hepatic pathogenesis, *World J Gastroenterol*, 2010, **16**(48), 6035-6043.
- Ahmad I, Aqil F and Owais M, Modern Phytomedicine: Turning Medicinal Plants into Drugs, KGaA, Weinheim, 2006.
- Thyagarajan S P, Jayaram S, Gopalakrishnan V, Hari R, Jeyakumar P and Sripathi M S, Herbal medicines for liver diseases in India, *J Gastroen Hepatol*, 2002, **17**(S3), S370-376.
- Pradeepa K, Krishna V, Venkatesh, Santosh Kumar S R and Rajesh K P, Antioxidant and prophylactic effects of *Delonix elata L.* stem bark extracts and flavonoid isolate quercetin against carbon tetrachloride induced hepatotoxicity in rats, *BioMed Res Int*, 2014, ID 507851.
- Chitra M, Sathyapriya R, Gayathiri U and Hema H, *In vitro* antioxidant activity of *Delonix elata L.*, *Asian J Pharm Health Sci*, 2011, **1**(4), 221-224.
- Manjunatha B K, Krishna V and Pullaiah T, Flora of Davanagere district, Karnataka, India, Regency Publications, New Delhi, 2004.
- Adeneye A A, Ajagbonna O P, Adeleke T I and Bello S O, Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats, *J Ethnopharmacol*, 2006, **105**, 374-379.
- Mohamed Khadeer A B, Krishna V and Chethan J D, *In vitro* antioxidant and *in vivo* prophylactic effects of two γ -lactones isolated from *Grewia tiliaefolia* against hepatotoxicity in carbon tetrachloride intoxicated rats, *Eur J Pharmacol*, 2010, **631**, 42-52.
- Beauchamp C and Fridovich I, Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels, *Anal Biochem*, 1971, **44**, 276-287.
- Aebi H, Catalase *in vitro*, *Methods Enzymol*, 1984, **105**, 121-126.
- Mohandas J, Marshall J J, Duggin G G, Horvath J S and Tiller D J, Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer, *Cancer Res*, 1984, **44**, 5086-5091.
- Warholm M, Guthenberg C, Bahr C V and Mannervik B, Glutathione transferase from human liver, *Methods Enzymol*, 1985, **113**, 499-504.
- Ohkawa H, Ohishi N and Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal Biochem*, 1979, **95**, 351-358.
- Galigher A E and Kayloff E N, Essential of practical microtechnique, Lea and Febiger, Philadelphia, 1971.
- Braca A, Tommasi N D, Bari L D, Pizza C, Politi M and Morelli I, Antioxidant principles from *Bauhinia terapotensis*, *J Nat Prod*, 2001, **64**, 892-895.
- Klein S M, Cohen G and Cederbaum A I, Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system, *Biochem*, 1991, **20**, 6006-6012.
- Nishimiki M, Rao N A and Yagi K, The occurrence of superoxide anion in the reaction of reduced phenazinemetosulfate and molecular oxygen, *Biochem Biophys Res Commun*, 1972, **46**, 849-853.
- Marcocci L, Packer L, Sckaki A and Albert G M, Antioxidant action of *Ginkgo biloba* extract EGB 761, *Methods Enzymol*, 1994, **234**, 462-475.
- Dinis T C P, Madeira V M C and Almeida M L M, Action of phenolic derivatives (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers, *Arch Biochem Biophys*, 1994, **315**, 161-169.
- Halliwell B and Gutteridge J M C, Free Radicals in Biology and Medicine, 4th Edn, Oxford University Press, Oxford, 2007.
- Rubinstein D, Epinephrine release and liver glycogen levels after carbon tetrachloride administration, *Am J Physiol*, 1962, **203**, 1033-1037.
- Recknagel R O, Glende Jr. E A, Dolak J A and Waller R L, Mechanisms of carbon tetrachloride toxicity, *Pharmacol Ther*, 1989, **43**, 139-154.
- Kadiiska M B, Gladen B C, Baird D D, Dikalova A E, Sohler R S, Hatch G E, *et al.*, Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl₄ poisoning?, *Free Radic Biol Med*, 2000, **28**, 838-845.
- Jia X Y, Zhang Q A, Zhang Z Q, Wang Y, Yuan J F, Wang H Y, *et al.*, Hepatoprotective effects of almond oil against carbon tetrachloride induced liver injury in rats, *Food Chem*, 2011, **125**, 673-678.
- Ranawat L, Bhatt J and Patel J, Hepatoprotective activity of ethanolic extracts of bark of *Zanthoxylum armatum* DC in CCl₄ induced hepatic damage in rats, *J Ethnopharmacol*, 2010, **127**, 777-780.
- Kew M C, Serum aminotransferase concentration as evidence of hepatocellular damage, *Lancet*, 2000, **355**, 591-592.
- Esterbauer H, Schaur R J and Zollner H, Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, *Free Radic Biol Med*, 1991, **11**, 81-128.
- Augustyniak A, Wazkilwicz E and Skrzydlewaka E, Preventive action of green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol, *Nutrition*, 2005, **21**, 925-932.