Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF *VERNONIA CINEREA* EXTRACT AGAINST CCL4 INDUCED HEPATOTOXICITY IN ALBINO RATS

G. Leelaprakash[1]*, S. Mohan Dass[2], V. Sivajothi[3]
1. Department of Biochemistry, Administrative Management College, 18th km, Bannerghatta road, Kalkere, Bangalore, India.
2. Kaamadhenu Arts and Science College, Sathyamangalam, Tamil Nadu-638 503, India.
3. Department of Pharmaceutical Chemistry, The Oxford College of Pharmacy, Bangalore, India.
*Corresponding author's E-mail: lpleelaprakash@gmail.com

Accepted on: 11-06-2011; **Finalized on:** 25-09-2011.

ABSTRACT

In the present study, we investigated the protective effect of *Vernonia cinerea* (F. Asteraceae) against carbon tetrachloride (CCl4) induced hepatotoxicity in albino rats. Hepatotoxicity was successfully induced by injecting CCl4 (0.5ml/kg body weight) intraperitoneally. Ethanol extract of *Vernonia cinerea* (250mg/kg) administrated intraperitoneally along with CCl4 for 7 days. The general observation, mortality, biomarker enzymes like lactate dehydrogenase (LDH), alkaline phospatase (ALP), diagnostic enzyme markers like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and antioxidants such as glutathione (GSH), Vit-C, glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) were monitored after 7 days of the last dose. CCl4 caused liver damage as evident by statistically significant ($p<0.001$) increased in plasma activities of AST, ALT, ALP, LDH. There were general statistically significant losses in activities of SOD, CAT, GPx, GSH, Vit-C, and an increase in MDA in the liver of CCl4-treated group compared with the control group. However, *Vernonia cinerea* were able to counteract these effects. The present results suggest that the plant can act as hepatoprotective against CCl4 toxicity and that the mechanism by which they exert hepatoprotection modulating antioxidant status.

Keywords: *Vernonia cinerea*, Antioxidant, Hepatotoxicity, Carbon tetrachloride, Lipid Peroxidation, Liver Marker Enzymes.

INTRODUCTION

Humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other undesirable contaminants in the air, food and soil. Most of these chemicals induce a free radical- mediated lipid peroxidation leading to disruption of biomembranes and dysfunction of cells and tissues. Therefore lipid peroxidation is a crucial step in the pathogenesis of free radical-related diseases including inflammatory injury and hepatic dysfunctions. It is also thought that antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by preventing free radical formation[1].

The free radical-mediated hepatotoxicity can be effectively managed upon administration of such agents possessing antioxidants[2], free radical scavengers[3] and anti-lipid peroxidation[4, 5] activities. Apart from the natural antioxidant defense system, there are various synthetic antioxidants in use but these compounds have been reported to have various side effects. In this context natural compounds isolated from plants deserve significance.

Carbon tetrachloride (CCl4) is a potent hepatotoxic agent causing hepatic necrosis, and is widely used in animal models for induction of acute and chronic liver injury. It also induces hydropic degeneration, fatty changes, cirrhosis and hepatoma. CCl4-induced hepatotoxicity is believed to involve two phases. The initial phase involves the metabolism of CCl4 by cytochrome P450 to the trichloromethyl radicals ($CCl_3^{\bullet}$ and/or CCl3OO), which lead to membrane lipid peroxidation and finally to cell necrosis[6]. The second phase of CCl4-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators[7].

It has been reported that treatment with CCl4 in mice and rats caused the release of AST and ALT, hepatocellular necrosis, decreased levels of antioxidative enzymes and increased lipid peroxidation products. The hepatotoxic effect induced by CCl4 could be reduced by treatment with dietary phytochemicals and antioxidants such as silymarin, flavonoids, curcumin, vitamin C and E[8]. In recent years, many researchers have become increasingly interested in herbal and edible plant extracts that possess hepatoprotective activities.

Vernonia cinerea (F. Asteraceae) is a common weed throughout India and it is well known as "Sahadevi" (Sanskrit), Naichette (or) Mukuthipundu. It has many therapeutic uses in different traditional medicine of the world. Different parts of the plant are of different therapeutic values. To mention a few, the plant is used for malaria fever, worms, pain, inflammation, infections, diuresis, cancer, abortion, and various gastro-intestinal disorders[9]. Other *Vernonia* species that shared some of these therapeutic values include: *Vernonia brachycalyx*, *Vernonia brasiliana*, *Vernonia herbacea, Vernonia subligera* and *Vernonia coloralia.*

In traditional system of medicine the whole plant with its small flowers is used medicinally to promote perspiration in febrile conditions. Co-administered with quinine it is beneficial in malarial fevers. Poultice of the leaves is useful against guinea worms. Flowers are administered for conjunctivitis. The flower extract of the plant was used in adjuvant-induced arthritis. The present study has been designed to evaluate the hepatoprotective effect of *Vernonia cinerea* in CCl4 induced albino rats.

MATERIALS AND METHODS

Plant

The leaves of *Vernonia cinerea* were collected from Trivandrum, Kerala, India, during December 2009. The plant was authenticated by Botanical Survey of India, Coimbatore, India. The leaves were shade dried at room temperature and subjected to size reduction to a coarse powder by using dry grinder and passed through a sieve. It was extracted to exhaustion with ethanol using a shaker. The extract thus obtained was dried using a rotary evaporator under reduced pressure at 40^oC.

Animal

The female wistar strains albino rats weighing between 150-180gm were obtained from KMCH Pharmacy College, Coimbatore. Animals were kept in animal house at an ambient temperature of 25^oC and 45-55% relative humidity, with 12h each of dark and light cycles. They were fed with standard rodent pellet, trade name "Gold Mohur rat feed" (Hindustan lever Ltd, India) and tap water ad libitum. The study was conducted after obtaining clearance from Institutional animal ethical committee (Bio.Chem/3/2005).

Chemicals

All the chemicals were obtained from Loba chemie Pvt. Ltd, Mumbai and S.D. Fine Chemicals, Chennai.

Induction of experimental hepatotoxicity

The liver injury was induced by CCl4 according to methods described previously[10, 11]. Liver damage was induced in rats with a 1:1 (v: v) mixture of CCl4 and olive oil, administered intraperitoneally at a dose of 0.5 ml/kg body weight for 7 days.

Experimental set up

The rats were randomly divided into four groups of six animals in each.

Group I: Served as control

Group II: Administered CCl4 (0.5ml/kg/bw) for 7 days.

Group III: Administered CCl4 (0.5ml/kg/bw) + Silymarin for 7 days.

Group IV: Received simultaneously both *Vernonia cinerea* (250mg/kg/bw) and CCl4 (0.5ml/kg/bw)7 days.

At the end of the experiment period, the animals in different groups were sacrificed by cervical decapitation. Blood and tissue were collected for various examinations.

In vitro Antioxidant Assay

DPPH radical scavenging activity

The free radical scavenging activity was determined by the method of Shimada *et al.* (1992) and Yang *et al*. (2006)[12, 13]. The ethanolic extracts were dissolved with ethanol to prepare various sample solutions at 10, 20, 30, 40, 50µg/ml. Each extract solution (2ml) was mixed with 1ml of methanolic solution containing DPPH radicals, with a final concentration of 0.2mM DPPH. The mixture was shaken vigorously and maintained for 30min in dark. The absorbance was measured at 517nm. The absorbance of the control was obtained by replacing the sample with methanol. Quercetin was used as standard reference. The scavenging activity was calculated using the formula, Scavenging activity (%) = [(A_{517} of control-A_{517} of sample) / A_{517} of control] X 100.

ABTS Radical scavenging activity

The ABTS radical scavenging activity of the extract was measured by Rice-Evans et al., (1997)[14]. Monocation radical $ABTS^+$ (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 hr before use. Different concentrations (10-50 µg/ml) of extract or standard (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with methanol to make 1 ml. The absorbance was read at 745nm and the % inhibition was calculated. The experiment was performed in triplicate.

Biochemical parameters

The following parameters were analysed to evaluate the hepatoprotective of *Vernonia cinerea* by the methods given below: Serum AST[15], ALT [16], ALP[17], and LDH[18] , a liver homogenate was used for the analysis of Superoxide dismutase[19], Catalase[20], glutathione peroxidase[21],Total reduced glutathione[22], ascorbic acid[23].

Determination of lipid peroxidation

MDA in liver homogenate and microsomal fraction was determined by the reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation[24].

Statistical analysis

All data were expressed as the means ± standard deviation (SD). Student's t- test was used to arrive at the statistically significant changes associated with various treatments. $p<0.05$ was regarded as significant.

RESULTS AND DISCUSSION

The liver is the major organ responsible for the metabolism of drugs and toxic chemicals, and therefore is the primary target organ for nearly all toxic chemicals[25]. The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years by using acute poisoning with CCl4[26]. CCl4 an extensively studied liver toxicant, and its metabolites such as trichloromethyl peroxy radical (CCl4 O_2^-) are known to be involved in the pathogenesis of liver damage[27].

In vitro antioxidant capacity of *Vernonia cinerea* extract, as assessed by DPPH & ABTS assay showed a dose-dependent activity. Which conform that the extract to possess antioxidant like properties, which may be a contributing factor for mitigation of CCl4 induced hepatotoxicity (Table 1).

Following CCl4 administration the level of all marker enzymes increased significantly in group II animals (p<0.001), as compared to normal controls (Table 2). *Vernonia cinerea* treatment caused a significant decreases (p<0.001) in the activities of all these enzymes in group IV animals. The increased activities of liver marker enzymes such as AST, ALT, ALP and LDH in the serum of CCl4 induced animals indicate damage to hepatic cells[28]. CCl4-mediated acute toxicity increased permeability of the hepatocyte membrane and cellular leakage[29]. The *Vernonia cinerea* mediated supression of the increased AST, ALT, ALP and LDH activities suggested the possibility of the extract to give protection against liver injury upon CCl4 induction.

Lipid peroxidation has been implicated in the pathogenesis of hepatic injury by compounds like CCl4 and is responsible for cell membrane alterations[30]. In the present study, significantly elevated level of lipid peroxides (*p*<0.001) observed in CCl4 administered rats (group II) indicated excessive formation of free radicals and activation of lipid peroxides system resulting in hepatic damage. The significant decline in the lipid peroxides content in the liver tissue of group IV rats indicated antilipid peroxidative effect of *Vernonia cinerea* (Table 3).

Table 1: Effect of *Vernonia cinerea* (VC) (% inhibition) on DPPH and ABTS radical scavenging activity

Concentration (µg/ml)	Quercetin	DPPH	ABTS
		Vernonia cinerea	
10	55.13±0.146	60.60±0.505	62.57±2.388
20	79.48±0.072	78.45±0.304	68.15±1.690
30	81.02±0.083	79.73±0.139	76.57±2.526
40	88.95±0.066	89.68±0.219	80.17±1.356
50	94.30±0.272	91.00±0.064	89.79±2.255
Ec-50(µg/ml)*	20.00	22.00	12.00

Each value was expressed as the mean ± SD. (n=3)

*Ec- 50 value was determined to be the effective concentrations at which DPPH; ABTS were scavenged by 50%, respectively. The Ec-50 value was obtained by interpolation from linear regression analysis.

Table 2: Effect of *Vernonia cinerea* (VC) on liver marker enzymes in the serum of control and experimental animals.

Parameters	Normal (group I)	Ccl4 induced (group II)	Ccl4+silymarin (group III)	Ccl4+VC (group IV)
ALT(IU/dl)	30.99±0.37	174.41±12.63♦	55.52±3.47*	76.02±3.74*
AST(IU/dl)	118.39±5.98	568.72±5.24♦	347.88±7.81*	364.84±4.65*
ALP(IU/dl)	117.39±2.10	343.44±7.56♦	211.29±11.22*	250.55±11.48*
LDH(IU/dl)	101.07±5.92	153.25±27.01♦	117.19±7.22*	127.19±12.2*

Values are mean±SEM for 6 animals in each observation

♦ p<0.001 as compared with normal group; * p<0.001as compared with CCl4 induced group

ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

Table 3: Effect of *Vernonia cinerea* (VC) on the antioxidant status of liver in the control and experimental animals.

Parameters	Normal (group I)	Ccl4 induced (group II)	Ccl4+ silymarin (group III)	Ccl4+VC (group IV)
SOD	2.04±0.05	1.75 ±0.05♦	2.02±0.08 *	1.99±0.13*
CAT	205.896±0.737	131.556±2.128♦	203.070±1.317*	205.050±0.827*
GP_X	11.375±0.452	5.183±0.087♦	11.083±0.256*	11.251±0.008*
GSH	49.831±0.229	27.262±0.311♦	49.758±1.317*	47.949±0.784*
Vit-C	39.831±0.119	37.610±0.400♦	39.413±0.292*	38.993±0.156*
LP	0.216±0.001	0.263±0.000♦	0.213±0.003*	0.217±0.003*

Values are mean±SEM for 6 animals in each observation

♦ p<0.001 as compared with normal group; * p<0.001as compared with CCl4 induced group

Values are expressed as SOD, superoxide dismutase (unit/min/mg protein); CAT, catalase (µmol/min/mg protein); GP_X, glutathione peroxidase (µg of glutathione consumed/min/mg of protein); GSH, glutathione (µg/mg protein); Vit-C, Vitamin-C (µg/mg protein); LP, lipid peroxidation (µmoles of malonaldehyde (MDA) formed/mg protein/h).

SOD, CAT, GSH, GP_X and Vit-C constitute a mutually supportive team of defence against reactive oxygen species (ROS). SOD (metalloprotein) is the first enzyme involved in the antioxidant defense by lowering the steady state of O_2^-. CAT is a hemoprotein, localized in the peroxisomes and catalyses the decomposition of H_2O_2 to water and oxygen. GPx, a selenoenzyme, present predominantly in liver and catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide[31].

CONCLUSION

In the present study pretreatment with *Vernonia cinerea* (group IV) showed increased activity of antioxidant enzymes compared to CCl4 treated animals indicating the potentiality of *Vernonia cinerea* to act as an antioxidant by preventing the peroxidative damage caused by CCl4.

REFERENCES

1. Sheweita SA, Abd El-Gabar M, Bastawy M. Carbon tetra chloride changes the activity of cytochrome P450 system in the liver of male rats: role of antioxidants. *Toxicology* 169: 2001, 83.
2. Attri S, Rana SV, Vaiphei K, Sodhi, CP, Katyal R, Nain RC, Goel CK, Singh K. Isoniazid- and rifampicin-induced oxidative hepatic injury—protection by *N*-acetylcysteine. *Hum Exp Toxicol* 19: 2000, 517.
3. Sadanobu S, Watanabe M, Nakamura C, Tezuka M, In vitro tests of 1,3-dithia-2 thioxo-cyclopent-4-ene to evaluate the mechanisms of its hepatoprotective action. *J Toxicol Sci* 24: 1999, 375.
4. Lim HK, Kim HS, Choi HS, Oh S, Jang CG, Choi J, Kim SH, Chang MJ. Effects of acetylbergenin against dgalactosamine-induced hepatotoxicity in rats. *Pharmacol Res* 42: 2000a,471.
5. Lim HK, Kim HS, Choi HS, Oh S, Choi J. Hepatoprotective effects of bergenen, a major constituent of *Mallotus japonicas* on carbon tetra chloride intoxicated rats. *J Ethnopharmacol.* 72: 2000b, 469.
6. Manibusan MK, Odin M, Eastmond DA. Postulated carbon tetrachloride mode of action: a review. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 25: 2007, 185–209.
7. Planagum A, Claria J, Miquel R, Lopez-Parra M, Titos E, Masferrer JL, Arroyo V, Rodes J. The selective cyclooxygenase-2 inhibitor SC-236 reduces liver fibrosis by mechanisms involving non-parenchymal cell apoptosis and PPAR gamma activation. *FASEB J* 19: 2005, 1120–1122.
8. Vitaglione P4, Morisco F, Caporaso N. Fogliano V. Dietary antioxidant compounds and liver health. *Critical Reviews in Food Science and Nutrition* 44: 2004,575–586.
9. Grainger CR. Medicinal plants of seychelles. *Journal of Royal Society of Health* 116:1996, 107–109.
10. Miyazawa T, Suzuki T, Fujimoto K, Kaneda T.Phospholipid hydroperoxide accumulation in liver of rats intoxicated with carbon tetrachloride and its inhibition by dietary alpha-tocopherol. *Journal of Biochemistry* 107: 1990, 689–693.
11. Yoshitake I, Ohishi E, Sano J, Mori T, Kubo K. Effects of KF-14363 on liver fibrosis in rats with chronic liver injury induced by carbon tetrachloride. *Journal of Pharmacobio-Dynamics* 14: 1991, 679–685.
12. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agriculture and Food Chemistry* 40: 1992, 945–948.
13. Yang B, Wang JS, Zhao MM, Liu Y, Wang W, Jiang YM. Identification of polysaccharides from pericarp tissues of litchi (Litchi chinensis Sonn.) fruit in raltion to their antioxidant activities. *Carbohydrate Research* 341: 2006, 634–638.
14. Rice-Evans C, Miller NJ, Papanga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci. Rev* 2(4): 1997 ,152-159.
15. Reitman S, Frankel S. Colorimetric method for the determination of serum oxaloacetic and glutamic pyrovic transaminase. *American Journal of Clinical Pathology* 28: 1957,56–63.
16. Jendrassik L, Groff P. Vereinfachte photometrische zur bestimmung des Blutbilirubins. *Biochemische Zeitschrift* 297: 1938, 81– 89.
17. King E J, Armstrong A R. *Canad Med Assn J* 31: 1934,376.
18. King J. In. *practical clinical enzymology.* Van,D(Eds). Norstand Company limited, London, 1965, 83-93.
19. Kakkar P, Das B, Viswanathan PN. *Indian Journal of Biochemistry and Biophysics* 21: 1984, 130-132.
20. Sinha AK. "Colorimetric Assay of Catalase". *Analytical Biochemistry* 47: 1972, 389-394.
21. Rotruck JT, Pope AL, Ganther HE, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 179: 1973, 588–590.
22. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione – s-transferase activities in rat lung and liver. *Biochem Biophys Acta* 582: 1979, 67–78.
23. Omaye, S.T, Turnbull, J.D, and Sauberlich, H.E. Selected Methods for the Determination of Ascorbic Acid in Animal Cells, Tissues, and Fluids, *Methods Enzymol* 62: 1979,3-11.
24. Ohkawa H, Ohishi W, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95: 1979,351–358.
25. Bissel DM, Gores GJ, Laskin DL, Hoorhagle JH. Drug-induced liver injury: mechanisms and test systems. *Hepatology* 33: 2001,1009–13.
26. Recknagel RO, Glende EA Jr, Dolak JA, Walker RL. Mechanisms of carbon tetrachloride toxicity. *Pharmacol Ther 43:* 1989,139-54.
27. Mehandale HM, Roth RA, Gandolfi AJ, Klaunig JE, Le-masters JJ, Curtis LR.Novel mechanisms in chemically induced hepatotoxicity. *FASEB J 104* : 1986, 826-9.
28. Wolf PL. Biochemical diagnosis of liver disease. *Indian J Clin Biochem 14* : 1999, 59-65.

29. Paduraru I, Saramet A, Danila Gh, Nichifor M, Jerca L, Iacobovici A, *et al*. Antioxidant action of a new flavonic derivative in acute carbon tetrachloride intoxication. *Eur J Drug Metab Pharmacokinet 21:* 1996,1-6.

30. Bandyopadhyay U, Das D, Banerjee RK. Reactive oxygen species: oxidative damage and pathogenesis. *Curr Sci 77:* 1999, 658-65

31. Venukumar MR, Latha MS, Antioxidant activity of *Curculigo orchioides* in carbon tetra chloride induced hepatopathy in rats. *Ind J Clin Biochem* 17: 2002, 80.

About Corresponding Author: Mr.G.Leelaprakash

Mr.G.Leelaprakash working as a Sr. Lecturer in the PG Department of Biochemistry, Administrative Management College, Bangalore. He is having 9 years of teaching experience in PG Department. He completed Master Degree in University of Madras and Master of Philosophy in Bharathidasan University, Tiruchirappalli. Now he is pursuing Doctor of Philosophy in Biochemistry, Bharathiar University, Coimbatore. His Research area is "Role of Medicinal plants in Cancer Biology".