ORIGINAL ARTICLE

In vitro antioxidant and hepatoprotective potential of *chenopodium album* extract

Ashish Baldi, Naveen Kumar Choudhary
Department of Phytopharmacology, Mandsaur Institute of Pharmacy, Mandsaur, Madhya Pradesh, India

Introduction: *Chenopodium album* (Bathua), a native Indian herb, has been used for treatment of abdominal pain, eye disease, throat troubles and cardiovascular disorders. The present study was carried out to explore antioxidant and hepatoprotective efficacy of *C. album* extract against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. **Materials and Methods:** The ethanolic extract of *C. album* seeds was evaluated for *in vitro* antioxidant activity using three established models (diphenyl picryl hydrazyl radical scavenging method, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation decolorization assay and nitric oxide radical scavenging method) and hepatoprotective activity was also assessed against CCl_4 induced hepatic damage in rats at the doses of 300 mg/kg and 450 mg/kg. **Results:** *C. album* extract was found to exhibit excellent antioxidant and free radical scavenging activity, when compared with ascorbic acid during *in vitro* studies. *C. album* extract at a dose of 450 mg/kg showed inhibition of elevation of the biochemical parameters comparable with silymarin (100 mg/kg) and hence shown remarkable protection in hepatic injury induced by free radicals generated due to administration of CCl_4 as toxicant. The observations were also supplemented with histopathological examination of rat liver sections. **Conclusion:** This study establish scientific evidences to folklore use of *C. album* in treatment of hepatic disorders and also suggests that free radical scavenging and antioxidant activities of phytoconstituents may be the possible mechanisms of its hepatoprotective potential.

Key words: 2,2' - azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), carbon tetrachloride, diphenyl picryl hydrazyl, free radical scavenging, nitric oxide, silymarin

INTRODUCTION

The importance of free radicals and reactive oxygen species (ROS) has attracted increasing attention of researchers involved in the field of medical science. Production of ROS is normal but essential process during cell metabolism to carry out important physiological functions.[1] Yet, overproduction and imbalance between production and utilization of free radicals results in oxidative stress. ROS including free radicals such as superoxide anion, hydroxyl, non-free radicals like H_2O_2 and singlet oxygen along with various forms of free oxygen species are involved in hepatocellular injury and related disorders, chronic degenerative diseases, inflammation, cataract, atherosclerosis, rheumatism, arthritis and ischemia etc.[2,3]

Prolonged exposure to certain xenobiotic, pollutants, long term drug therapy, excessive use of some of the commonly used medicines like paracetamol, diclofenac, etc., alcoholism, and certain disease state have been reported to affect liver functioning. The major clinical manifestation of liver disorder is jaundice. Despite of the excellent regeneration capacity of this organ, a slight injury or toxicity may lead to fatal complications. Therefore, being a vital organ, its protection has a special status in therapeutics.[4]

Liver, an important organ actively involved in metabolic functions, is a frequent target of number of toxicants.[5] Modern allopathic treatment does not hold promise to cure liver disease perfectly. Several synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene are available, but are quite unsafe and their toxicity is a problem of concern.[6] However, traditional system of Indian medicines, i.e., Ayurveda recommended a number of medicinal preparations for the treatment of liver disorders based on indigenous plants and their extracts.[7] Therefore, natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention. Foods rich in antioxidants offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanisms and thus prevent and cure liver damage.[8] Thus, the efficacy of the drug would be preventive and passive for defending against damages.[9]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.111614

Address for correspondence: Dr. Ashish Baldi, Mandsaur Institute of Pharmacy, Rewas Dewra Road, Mandsaur - 458 001, Madhya Pradesh, India. E-mail: baldiashish@gmail.com
Received: 26-12-2011; **Accepted:** 15-02-2013

Carbon tetrachloride (CCl_4) is a widely used experimental hepatotoxicant to study hepatoprotective potential of test drugs/preparation as the changes associated with CCl_4 induced liver damage has been appeared very similar to the acute viral hepatitis.[10] The principle causes of CCl_4 induced hepatic damage are lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals.[11,12] The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against hepatic damage.[9]

In recent years, a number of plants have been shown to possess hepatoprotective property by improving antioxidant status but absence of detailed and systematic scientific studies for scientific validation of their therapeutic potential remains a major area of concern for their acceptance by pharmaceutical industries.[13] Therefore, present study was carried out to explore antioxidant and hepatoprotective efficacy of *Chenopodium album* extract against CCl_4 induced hepatotoxicity in animal model.

C. album, commonly known as Bathua or Goose foot, is well distributed in India and usually found as weed in early grain fields in Madhya Pradesh, Rajasthan, Punjab, Kashmir, Sikkim, Bengal, and Mumbai.[14] The plant mainly contains flavonoids, alkaloids and tannins and is reported to improves appetite, have laxative, diuretic, anthelmintic effect. The plant is also known to be useful in abdominal pain, eye disease, throat troubles and cardiovascular disorders.[15] Several flavonoids and alkaloids are reported to possess antioxidant and hepatoprotective properties.[16] Hence, present investigation was undertaken to determine the antioxidant and hepatoprotective potential of *C. album* seeds.

MATERIALS AND METHODS

Plant Materials

The seeds of *C. album* were collected from local field of Mandsaur (M.P.) and authenticated by Dr. Rakesh Gupta, Department of Dravyaguna, Smt. Dhairya Prabha Devi Sojatia Ayurved Medical College, Bhanpura. Voucher specimen was deposited to herbarium of SDPS Ayurved Medical College, Bhanpura vide specimen no. SDPS/09/PS/114.

Extraction

The air dried seeds were powdered in an end runner mil for 18 h. The powder (250 g) was passed through 100 mesh size sieve and defatted with petroleum ether (60-80°) for 48 h. The defatted powder was allowed to dry at 25 ± 2°C in order to remove traces of petroleum ether. The defatted seeds powder was then subjected to continuous extraction using soxhlet apparatus with ethanol (95% v/v) as a menstrum. After which, ethanol was distilled off and the extract so obtained was concentrated and dried under reduced pressure. The dried extract was 5.8% w/w.

Phytochemical Study

The extract was analyzed for presence of various phytochemicals viz. carbohydrates, alkaloids, glycosides, phenolics and flavanoids by performing qualitative analysis.[17]

Animal Studies

Institutional Animal Ethical Committee (IAEC) of the institute approved the study protocol (Mandsaur Institute of Pharmacy/IAEC/11/1019/C/06/010) and was in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals. Three month old Wistar Albino rats (150-200 g) of either sex were obtained from Animal House, Mandsaur Institute of Pharmacy, Mandsaur (M.P.). They were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity (50 ± 15%) and normal photoperiod (12-h light/dark cycle) were used for the experiment. Commercial pellet diet (Nav Maharastra Chakan Oil Mills Ltd., New Delhi, India) and water were provided *ad libitum* throughout the course of study.

For induction of hepatic injury, CCl_4 was obtained from Suvidhinath Lab, Baroda and standard hepatoprotective drug, silymarin, was procured from Microlabs Ltd., Bengaluru, Karnataka.

Antioxidant Activity

The antioxidant activity was determined by three established methods, diphenyl picryl hydrazyl (DPPH) radical scavenging method, ABTS radical cation decolorization assay and nitric oxide (NO) radical scavenging method.

DPPH radical scavenging activity was measured by spectrophotometric method.[18] For the present study, the samples were prepared in different concentrations, i.e., 5-100 µg/ml in methanol. The ethanolic extract of *C. album* (samples) at above concentrations was mixed with 3 ml of 100 µM DPPH prepared in methanol and final volume was made up to 4 ml with methanol. The absorbance of the resulting solutions was recorded in triplicate after 20 min at 25 ± 2°C against ascorbic acid. The disappearance of color was read spectrophotometrically at 517 nm using a Shimadzu visible spectrophotometer. Percent scavenging was calculated by following equation:

% Scavenging = 100 × [Absorbance (Blank) − Absorbance (Sample)/Absorbance (Blank)]

From obtained Radical Scavenging Capacity (RSC) values, the half maximal inhibiting concentration (IC_{50}) were

calculated, which represents concentration of scavenging compound that caused 50% neutralization.

ABTS radical cation (ABTS+) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulfate and the mixture was allowed to stand in dark at 25 ± 2°C for 12-16 h before use. For this study, different concentrations (5-100 µg/ml) of the ethanolic extracts (2 ml) were added to 1.2 ml of ABTS solution and final volume was made up with ethanol to 4 ml. The absorbance was read at 745 nm and the experiment was performed in triplicate.[19]

NO was generated from sodium nitroprusside and measured by Griess' reaction.[20,21] Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations (5-100 µg/ml) of ethanolic extracts dissolved in phosphate buffer saline and the tubes were incubated at 25 ± 2°C for 5 h. Control experiments without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess' reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylenediamine was recorded at 546 nm. The experiment was repeated in triplicate.[22]

Hepatoprotective Activity

The CCl_4 induced hepatotoxic rat model was used to determine the hepatoprotective activity of *C. album* extract.[23] The rats were divided into the following five groups each containing 6 rats ($n = 6$):

Group I: Control rats: Tween 80 in distilled water 1% v/v (5 ml/kg, p.o.) for 7 days

Group II: Toxic control rats: Administered vehicle (5 ml/kg p.o.) daily + CCl_4 in olive oil 1:1 v/v (0.7 ml/kg, ip) on alternate days for 7 days

Group III: Reference rats: Treated with silymarin 100 mg/kg + CCl_4 in olive oil 1:1 v/v (0.7 ml/kg, ip) on alternate days for 7 days

Group IV: *C. album* treated rats: Received ethanolic extract of *C. album* seeds 300 mg/kg body weight p.o. + CCl_4 in olive oil 1:1 v/v (0.7 ml/kg, ip) on alternate days for 7 days

Group V: *C. album* treated rats: Treated with ethanolic extract of *C. album* seeds 450 mg/kg body weight p.o. + CCl_4 in olive oil 1:1 v/v (0.7 ml/kg, ip) on alternate days for 7 days.

After 24 h of last treatment, rats were anesthetized with ether and blood samples from each animal of all groups were collected by retro-orbital plexus puncture in sterilized centrifuge tubes and the rats were then dissected to isolate liver. The blood samples were then allowed to coagulate at 30°C for 45 min and serum portion was separated from each sample by centrifugation at 25,000 g at 30°C for 10 min and subjected to biochemical investigation to assess liver function on the basis of total bilirubin, serum aminotransferase (alanine and aspartate) and alkaline phosphatase.[24] Total protein was estimated as per the method of Lowry *et al.*[25]

Statistical Analysis

The results are expressed as means ± standard deviation (SD) and values were calculated for each group. A one way analysis of variance (ANOVA) followed by Dunnet's test for significance analysis using Graph Pad Prism software. The minimum level of significance was set of $P < 0.05$.

RESULTS

The results of preliminary phytochemical screening revealed the presence of glycosides, phenolic compounds and flavonoids.

Alcoholic extract of *C. album* seeds in graded concentrations (5, 25, 50, 75 and 100 µg/ml) was tested for antioxidant activity in three different *in vitro* models. It was observed that the test compounds scavenged free radicals in a concentration dependent manner in the models studied. Maximum percentage inhibition of DPPH by the extract was 38.78% at 100 µg/ml concentration [Table 1]. Standard drug, i.e., ascorbic acid showed 48.59% inhibition of the DPPH radical at 50 µg/ml.

In ABTS radical cation method, maximum absorbance of *C. album* extract at 100 µg/ml level was comparable

Table 1: Antioxidant activity of ethanolic extract of *Chenopodium album#**

Concentration (µg/ml)	DPPH		ABTS		NO	
	C. album	Ascorbic acid	*C. album*	Ascorbic acid	*C. album*	Ascorbic acid
5	8.23±1.12	22.32±1.43	9.54±0.79	12.97±0.99	10.79±0.69	22.11±0.23
25	14.14±1.19	36.71±1.31	26.43±1.17	38.72±1.38	13.22±0.87	30.29±0.39
50	24.67±0.98	48.59±1.09	34.32±2.12	49.49±1.33	20.43±0.56	45.89±0.56
75	32.32±1.65	54.65±1.72	51.77±1.75	65.89±1.61	27.21±0.99	55.76±0.59
100	38.78±1.25	58.21±1.11	63.34±1.43	76.97±1.32	35.96±0.74	63.67±0.69

*The values given are mean of triplicate readings in different experiments, #All results are statistically significant with $P<0.05$ and values represents the mean±SEM; DPPH – Diphenyl picryl hydrazyl; NO – Nitric oxide; *C. album – Chenopodium album;* ABTS –2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

with ascorbic acid (75 µg/ml). The extract shows free radical scavenging activity in dose dependent manner as shown in Table 1. In NO model, maximum percentage inhibition of NO radicals by *C. album* extract was 35.96% at a concentration of 100 µg/ml [Table 1]. Ascorbic acid at 50 µg/ml level caused 45.89% inhibition.

In the hepatoprotective study, after treatment with CCl_4, a significant increase in levels of AST, ALT, ALP, total bilirubin and in liver weight, as compared to the normal control, were observed suggesting the liver toxicity and the decrease in total protein confirmed the liver toxicity. Levels of biochemical parameters were significantly lower in the rats pre-treated with silymarin. The groups of rats pre-treated with *C. album* extract, demonstrated dose dependent inhibition of elevation of the biochemical parameters. *C. album* extract, at a dose of 450 mg/kg, showed comparable inhibition of increase in biochemical parameters with reference to silymarin (100 mg/kg). Also the liver weight was significantly reduced in silymarin and *C. album* treated groups [Table 2].

Histopathological Studies

The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years by using acute poisoning with CCl_4. CCl_4 an extensively studied liver toxicant, and its metabolites such as trichloromethyl peroxy radical ($CCl_4.O_2^-$) are known to be involved in the pathogenesis of liver damage. It is bio-transformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca^{2+} haemostasis and finally result in cell death.[26]

The effect of *C. album* (300 and 450 mg/kg) and silymarin on liver histopathology of CCl_4 treated rats are presented in Figure 1. As seen in Figure 1a, liver section of normal rat showed distinct hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus. CCl_4 induced liver damage can be observed directly in Figure 1b, where the section showed massive fatty change, necrosis, lymphocyte infiltration, the loss of cellular boundaries, and join together of nucleus. Figure 1c presents the rat liver section treated with silymarin and CCl_4. In 300 mg/kg *C. album* and CCl_4 treated group, section suggested moderate degree of damage, with some fatty change, necrosis, and lymphocyte infiltration [Figure 1d]. Figure 1e shows the section of group treated with high dose *C. album* (450 mg/kg) and CCl_4, sections of these two groups were nearly comparable to the normal group, with no obvious necrosis was observed.

DISCUSSION

In the present study, three widely used methods for evaluation of antioxidant activity viz. DPPH, ABTS and NO assays were applied. *C. album* showed good radical scavenging activity against both DPPH and ABTS radicals. DPPH is a relatively stable free radical which when encounters proton donors such as antioxidants, it gets quenched and the absorbance decreases.[27] Results indicated definite scavenging activity of the extract towards DPPH radicals in comparison with ascorbic acid. The ABTS decolourization assay is based on the principle of inhibition/decrease in the absorbance of the radical cation (ABTS+). This chemical reaction results in direct generation of ABTS radical mono cation prior to addition of antioxidant components instead of in presence of antioxidant.

NO is a free radical produced in the mammalian cells and is involved in regulation of various physiological processes. However, excess production of NO is associated with several diseases like adjuvant arthritis, cancer etc.[28,29] NO free radical scavenging activity of the extracts was studied by using Griess reagent. *C. album* ethanolic extract was found to scavenge the NO free radical dose dependently.

C. album demonstrated dose dependent anti-oxidant activity comparable with ascorbic acid. Furthermore the results were presented in terms of % scavenging capacity as compared to initial concentration taken of free radicals. As the tests were carried out to investigate antioxidant potential of the extract at preliminary levels, i.e., maximally up to 100 µg/ml, increasing the concentration would surely resulted in

Table 2: Effect of ethanolic extract of *Chenopodium album* on biochemical parameters

Groups	Biochemical parameters					Liver weight (g)
	Aspartate aminotransferase (IU/l)	Alanine aminotransferase (IU/l)	Alkaline phosphatase (IU/l)	Total bilirubin (mg/dl)	Total protein (gm/dl)	
Control	45.20±1.24	144.6±0.42	156.2±1.58	0.53±0.05	7.37±0.18	5.96±0.18
Toxic control, carbon tetrachloride	243.5±2.70*	396.8±0.43*	388.4±9.4*	3.60±0.15*	4.49±0.09	9.62±0.32
Reference, silymarin (100 mg/Kg)	109.9±1.11#	204.2±1.27#	164.8±1.24#	1.15±0.08#	7.08±0.23	6.35±0.27
C. album, 300 mg/Kg	134.5±3.6#	258.2±1.46#	202.2±6.2#	2.6±0.17#	5.56±0.12	8.06±0.28
C. album, 450 mg/Kg	114.0±0.99#	230.5±2.12#	170.6±2.2#	1.62±0.04#	6.30±0.16	7.54±0.17

n=6; **P*<0.05 compared to normal; #*P*<0.05 compared to toxic control treated group; *C. album* – *Chenopodium album*

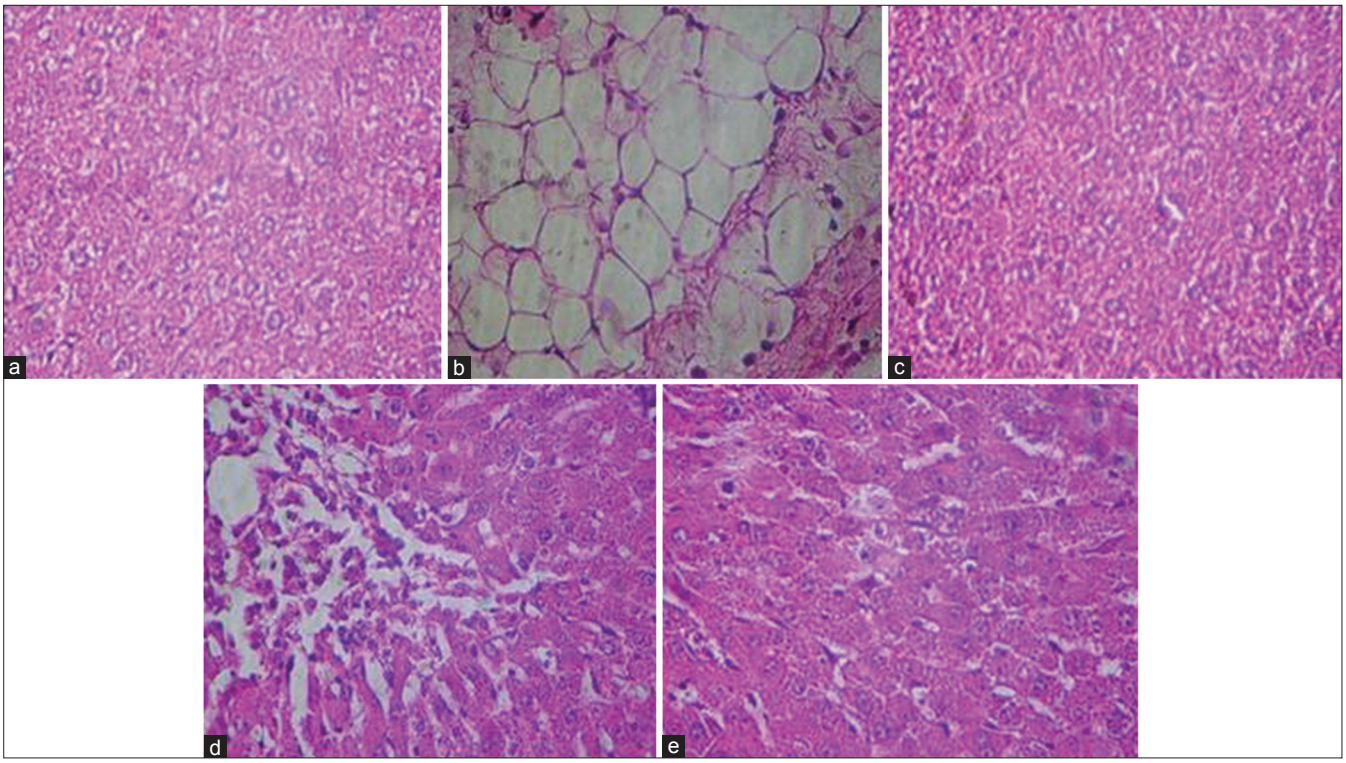

Figure 1: Effect of *C. album* and silymarin on the liver histopathology of the carbon tetrachloride treated rat. (a) Liver section of control rat; (b) Liver section of carbon tetrachloride treated rat, toxic control; (c) Liver section of silymarin (100 mg/kg) treated rat; (d) Liver section of rat treated with *C. album* (300 mg/kg) and (e) Liver section of rat treated with *C. album* (450 mg/kg)

greater scavenging potential. *C. album* extract used in present study had shown antioxidant potential in order of ABTS > DPPH > NO. The scavenging capacity of the extract was compared to ascorbic acid as standard. It was found that the IC_{50} values of *C. album* extract was 72.46 µg/ml and 143.63 µg/ml for ABTS and DPPH models, which were quite comparable with ascorbic acid having respective value of 50.77 µg/ml and 54.41 µg/ml, respectively.

CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver disease. The hepatotoxic effects of CCl_4 are largely due to generation of free radicals.[30] CCl_4 is metabolized to trichloromethyl free radicals by the cytochrome P450 system.[31] These free radicals then covalently bind to macromolecules of cell membranes and organelles to elicit lipid peroxidation, which will cause the loss of integrity of cell membrane.[32] Several plants, e.g. *Cordia macleodii*[16] and *Panax notoginseng*[33] have been tested for their efficacy in controlling the CCl_4 induced liver damage.

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and deplection in the tissue glutathione (GSH) levels. In addition, serum levels of many biochemical markers like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), cholesterol, bilirubin, alkaline phosphate are elevated. One of the earlier study on *Launaea intybacea* plant also revealed that aqueous extract significantly reduced serum bilirubin, SGOT, SGPT and alkaline phosphatase (ALP) levels and liver homogenates lipid peroxidase (LPO), superoxide dismutase (SOD), catalasae (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST) and increases GSH levels, suggesting hepatoprotective activity. Phytoconstituents like flavonoids and triterpenoids are known to possess hepatoprotective activity.[34] Our phytochemical study also reveals the presence of glycosides, flavonoids and phenolics. These suggest that the antioxidant activity and protective effects against CCl_4 induced hepatic cell injury of the extract could be due to the flavonoids, glycosides and phenolics present in *C. album*. Liver damage can be assessed by biochemical studies. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are present in high concentration in hepatic cells, which get released out into circulation due to the hepatic injury.[35] In present study, significant increase in the total bilirubin, AST, ALT and ALP activities in the CCl_4 treated group could be taken as an index of liver damage. Treatment with *C. album* extract inhibited CCl_4 induced liver damage by increase in total bilirubin, AST, ALT and ALP activities as compared with CCl_4 treated group. The CCl_4 induced a significant increase in liver weight, which is due to blocking of secretion of hepatic triglycerides in plasma.[36] Silymarin and the extract prevented the increase of liver weight in rats.

As observed in our experiment, administration of CCl_4 led to the elevation of total bilurubin, AST, ALT and ALP levels in serum and increase in weight of liver while decrease in total protein content, indicating significant liver damage. Pre-treatment with *C. album* had reversed these trends towards normalizations, reflecting that *C. album* possesses potent hepatoprotective activity *in vivo*. Polyphenols and glycosides present in the ethanolic extract of *C. album* were reported for excellent antioxidant potential.[37] A glycoside namely chenoalbuside from alcoholic extract of *C. album* was also reported to exhibit antioxidant potential.[38] The antioxidant activity of these phytoconstituents are mainly owing to their redox properties, i.e., the ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, could also be due to their metal chelation potential.[39] Phytoconstituents like flavonoids and phenolics are already known to possess hepatoprotective potential by inhibiting xenobiotic induced hepatotoxicity mainly due to their antioxidant or free radical scavenging activities.[39-42]

Furthermore, silymarin, a flavonoid from *Silybum marianum* was used as standard to study the hepatoprotective potential. The mechanism of action of this phytocompound is based on its antioxidant potential to a variety of free radicals. *C. album* extract used in present study had shown hepatoprotective potential as compared with silymarin on the basis of restoration of biochemical parameters such as AST, ALT, ALP, bilirubin and total protein content. On the basis of this and antioxidant potential of *C. album* extract against different kinds of free radicals as established during *in vitro* assays, it was presumed that the mechanism of hepato-protection was largely due to antioxidant potential of *C. album* extract. Histopathological observation of the liver tissue had directly supported this conclusion. Therefore, present work provides a conclusive scientific evidence for traditional use of *C. album* in treatment of hepatic disorders.

REFERENCES

1. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
2. Afonso V, Champy R, Mitrovic D, Collin P, Lomri A. Reactive oxygen species and superoxide dismutases: Role in joint diseases. Joint Bone Spine 2007;74:324-9.
3. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408:239-47.
4. Thomas SH. Paracetamol (acetaminophen) poisoning. Pharmacol Ther 1993;60:91-120.
5. Meyer SA, Kulkarni AP. Hepatotoxicity. In: Hodgson E, Smart RC, editors. Introduction to Biochemical Toxicology. New York: A. John Wiley and Sons, Inc; 2001. p. 487-90.
6. Madhavi DL, Salunkhe DK. Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK, editors. Food Antioxidants. New York: Marcel Dekker; 1995. p. 267.
7. Chatterjee TK. Medicinal plants with hepatoprotective properties. In: Herbal Options. 3rd ed. Calcutta: Books and Allied (P) Ltd; 2000. p. 135-7.
8. Miller NJ, Rice-Evans CA. Factors influencing the antioxidant activity determined by the ABTS.+ radical cation assay. Free Radic Res 1997;26:195-9.
9. Handa SS, Sharma A, Chakraborti KK. Natural products and plants as liver protecting drugs. Fitoterapia 1986;57:307-45.
10. Venukumar, MR, Latha MS. Hepatoprotective effect of the methanolic extract of orchids in Carbon tetrachloride treated male rats. Indian J Pharmacol 2002;34:269-75.
11. Castro JA, De Ferreyra EC, De Castro CR, De Fenos OM, Sasame H, Gillette JR. Prevention of carbon tetrachloride-induced necrosis by inhibitors of drug metabolism – Further studies on their mechanism of action. Biochem Pharmacol 1974;23:295-302.
12. Poli G. Liver damage due to free radicals. Br Med Bull 1993;49:604-20.
13. Mansour HH, Hafez HF, Fahmy NM. Silymarin modulates Cisplatin-induced oxidative stress and hepatotoxicity in rats. J Biochem Mol Biol 2006;39:656-61.
14. Kirtikar KR, Basu BD. Chenopodium (Tourn.) Linn. Indian Medicinal Plants. 2nd ed. Dehradun: International Books Distributor; 1999. p. 2072-4.
15. Nadkarni AK. *Chenopodium album* Linn. Indian Materia Medica. Mumbai: Popular Prakashan; 2002. p. 305.
16. Naseem N, Qureshi A, Bhanudansh S, Kuchekar B, Nadeem A, Logade A, *et al*. Antioxidant and hepatoprotective activity of *Cordia macleodii* leaves. Saudi Pharma J 2009;17:299-302.
17. Kokate CK, Purohit AP, Gokhale SB. Analytical Pharmacognosy Pharmacognosy. 5th ed. Pune, India: Nirali Prakashan; 1997. p. 109-37.
18. Rajakumar DV, Rao MN. Dehydrozingerone and isoeugenol as inhibitors of lipid peroxidation and as free radical scavengers. Biochem Pharmacol 1993;46:2067-72.
19. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and 15N nitrate in biological fluids. Anal Biochem 1982;126:131-8.
20. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. Biochem Biophys Res Commun 1994;201:748-55.
21. Baldi A, Panwar MS, Gupta R. Evaluation of *In vitro* antioxidant activity of *Eclipta alba*. Int J Pharma Biol Arch 2011;2:767-71.
22. Baldi A, Hussain W, Tailor Y. Evaluation of *in vitro* cultured cells of *Withania somnifera* for antioxidant activity. Curr Trends Biotechnol Pharm 2010;4:589-95.
23. Baheti JR, Goyal RK, Shah GB. Hepatoprotective activity of *Hemidesmus indicus R*. br. in rats. Indian J Exp Biol 2006;44:399-402.
24. Gupta AK, Mishra N. Hepatoprotective activity of ethanolic extract of *Chamomile capitula* in paracetamol intoxicated albino rats. Am J Pharmacol Toxicol 2006;1:17-20.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
26. Recknagel RO, Glende EA Jr, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 1989;43:139-54.
27. Wu HC, Chen HM, Shiau CY. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res Int 2003;36:949-57.
28. Ray G, Husain SA. Oxidants, antioxidants and carcinogenesis. Indian J Exp Biol 2002;40:1213-32.
29. Ialenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacol 1993;110:701-6.
30. Shenoy KA, Somayaji SN, Bairy KL. Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. Ind J Pharmacol 2001;33:260-6.

31. Jia XY, Zhang QA, Zhang ZQ, Wang Y, Yuan JF, Wang HY, *et al*. Hepatoprotective effects of almond oil against carbon tetrachloride induced liver injury in rats. Food Chem 2011;125:673-8.
32. Ranawat L, Bhatt J, Patel J. Hepatoprotective activity of ethanolic extracts of bark of *Zanthoxylum armatum* DC in CCl_4 induced hepatic damage in rats. J Ethnopharmacol 2010;127:777-80.
33. Yoshikawa M, Morikawa T, Kashima Y, Ninomiya K, Matsuda H. Structures of new dammarane-type Triterpene Saponins from the flower buds of *Panax notoginseng* and hepatoprotective effects of principal Ginseng Saponins. J Nat Prod 2003;66:922-7.
34. Baek NL, Kim YS, Kyung JS, Park KH. Isolation of anti-hepatotoxic agent from the roots of *Astragalus membranaceous*. Korean J Pharmacog 1996;27:111-6.
35. Kew MC. Serum aminotransferase concentration as evidence of hepatocellular damage. Lancet 2000;355:591-2.
36. Aniya Y, Koyama T, Miyagi C, Miyahira M, Inomata C, Kinoshita S, *et al*. Free radical scavenging and hepatoprotective actions of the medicinal herb, *Crassocephalum crepidioides* from the Okinawa Islands. Biol Pharm Bull 2005;28:19-23.
37. Kumar S, Chatterjee R, Dolai S, Adak S, Kabir SN, Banerjee S, *et al*. *Chenopodium album* seed extract-induced sperm cell death: Exploration of a plausible pathway. Contraception 2008;77:456-62.
38. Nahar L, Sarker SD. Chenoalbuside: An antioxidant phenolic glycoside from the seeds of *Chenopodium album* L. (Chenopodiaceae). Braz J Pharmacog 2005;15:279-82.
39. Kumarasamy Y, Byres M, Cox PJ, Delazar A, Jaspars M, Nahar L, *et al*. Isolation, structure elucidation and biological activity of flavones C-glycosides from the seeds of *Alliaria petiolata*. Chem Nat Comp 2004;40:122-8.
40. Akindele AJ, Ezenwanebe KO, Anunobi CC, Adeyemi OO. Hepatoprotective and *in vivo* antioxidant effects of *Byrsocarpus coccineus* Schum. and Thonn.(Connaraceae). J Ethnopharmacol 2010;129:46-52.
41. Alan L, Miller ND. Antioxidant flavonoids: structure, function and clinical usage. Alt Med Rev 1996;1:103-11.
42. Xiong X, Chen W, Cui J, Yi S, Zhang Z, Li K. Effects of ursolic acid on liver-protection and bile secretion. Zhong Yao Cai 2003;26:578-81.

How to cite this article: Baldi A, Choudhary NK. *In vitro* antioxidant and hepatoprotective potential of *chenopodium album* extract. Int J Green Pharm 2013;7:50-6.

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