Pharmacognosy Communications
Volume 3 | Issue 3 | Jul–Sep 2013
www.phcogcommn.org

Research Article

Antioxidative and hepatoprotective effect of *Corchorus depressus* L. against CCl_4-induced toxicity in rats

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Submission Date: 3-4-2013; Accepted Date: 14-4-2013

ABSTRACT: **Aims:** The study aimed to evaluate the antioxidant and hepatoprotective activity of an ethanolic extract from *Corchorus depressus* (CDEE). **Materials and Methods:** The total phenolic and flavonoid content of CDEE was determined using standard curve of gallic acid and quercetin respectively. *In vitro* antioxidant activity was determined by 2,2-diphenyl-1-picryl hydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) radicals and hydrogen peroxide methods. CDEE was administered orally (200 mg, 400 $mg.kg^{-1}.day^{-1}$) to rats for 7 days. Silymarin was used as positive control. On the 8th day, the rats were given a single dose of CCl_4 (1.0 ml/kg, i.p.) except vehicle control rats. After 24 h of CCl_4 administration, plasma markers of hepatic damage, hepatic antioxidants and indices of lipid peroxidation along with microscopic evaluation of liver were assessed in control and treatment groups. **Results:** The CDEE possessed strong antioxidant activity *in vitro*. Pre-treatment of CDEE to CCl_4 treated rats prevented hepatocyte damage as evidenced by estimating various biochemical parameters and histopathological observations. CDEE significantly prevented CCl_4 induced elevation of aspartate aminotransferase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and CCl_4 induced decrease in total protein (TP) level in Wistar rats. The CDEE treated rat liver anti-oxidant parameters superoxide dismutase (SOD), catalase (CAT), monodialdehyde (MDA) and glutathione (GSH) were significantly antagonized for the pro-oxidant effect of CCl_4. Histopathological studies also supported the protective effect of CDEE. **Conclusion:** This study partially validated the folk and traditional use of snail in liver disorder through CCl_4-induced rat experimental model.

KEYWORDS: ABTS, Antioxidant, *Corchorus depressus*, CCl_4, DPPH, Hepatoprotective

INTRODUCTION

Organisms need oxidation for the production of energy to fuel biological processes.[1] Yet overproduction of oxygen free-radicals causes lipid peroxidation of cellular membranes, proteins and DNA oxidation and results in hepatocyte injury.[2] To prevent the damage caused by oxidative stress, living organisms have developed an antioxidant defense system that includes the presence of nonezymatic antioxidants (e.g. glutathione, uric acid, bilirrubin, and vitamins C and E) and enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).[3] Based on the hypothesis that oxidative stress occurs only when the antioxidant capacity is insufficient to cope with the generation of prooxidants, many studies have focused on the changes in the liver antioxidants in hepatotoxicity.[4] It has been proposed that in addition to these natural antioxidant systems, other synthetic or natural reactive oxygen species (ROS) scavengers may reduce the incidence of free radical-mediated diseases. Synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate the scientific basis for traditional herbal medicines that are claimed to possess hepatoprotective activity.[5] Many plant extracts have been evaluated for hepatoprotective and antioxidant effects against carbon tetrachloride (CCl_4) induced hepatotoxicity

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DOI: 10.5530/pc.2013.3.5

model.[6–8] CCl_4 induced hepatotoxicity in rat is the common experimental *in vitro* model for the hepatoprotective drug screening. CCl_4 is a potent lipid-soluble hepato toxicgent which produces per oxidative degeneration of many tissues.[9] The toxicity of CCl_4 probably depends on the formation of the trichloromethyl radical ($CCl_3^{\cdot}$) which in the presence of oxygen forms the more toxic trichloromethyl peroxyl radical ($CCl_3O^{\cdot}$).[10] Lipid peroxidation is initiated by the interaction of these reactive radicals with unsaturated fatty acids of membrane lipids.[9,10]

Corchorus depressus (Linn.) (Family: Tiliaceae) commonly known as 'bhaufali' is perennial herb, woody 6–9 inches in length. The plant is regarded as good sand binder in the desert.[11] It is distributed in arid and semi-arid regions of India and Pakistan to North and tropical Africa. Medicinal uses of this plant in general weakness, gonorrhea, diabetes, treachery troubles, improved sexual vigor, jaundice have been reported.[12–14] Antipyretic and analgesic activities of a new triterpenic acid isolated from *Corchorus depressus* have been reported.[15,16] Ali and Ansari reported that application of the combined extracts of *E. officinalis*, *L. inermis*, *N. jatamansi* and *C. depressus* prepared in the oil of *Sesamum indicum* diminished the falling of hair and gave them the original color.[17] Chemical investigation of this plant has resulted in the isolation of various triterpenes,[18,19] phenolics and sterols.[20] According to Ayurvedic sources this plant is widely used as a hepatic tonic for many liver ailments. However, there are no systematic scientific reports in the modern literature regarding its usefulness as a hepatoprotective agent. To clarify this ethnopharmacological relevance, antioxidant and hepatoprotective effects of an ethanolic extract from whole plant of *Corchorus depressus* were studied in rats intoxicated with carbon tetrachloride (CCl_4).

MATERIALS AND METHODS

Plant materials

Corchorus depressus (Linn.) was collected from Jodhpur district of Rajasthan and identified from Botanical Survey of India, Arid Zone Regional Centre, Jodhpur-342 008 and a voucher specimen no. LMC/AP/001 was deposited in the college for future reference.

Preparation of extract

Corchorus depressus (whole plant) was dried under shade at room temperature. After drying, the plant was subjected to size reduction to a coarse powder by using dry grinder. The powder was packed into a Soxhlet apparatus and defatted with petroleum ether (60–80 °C). The marc was dried and extracted with ethanol at 80 °C for 24 h. The *Corchorus depressus* ethanol extract (CDEE) was concentrated to dryness under reduced pressure in a rotary evaporator and stored in airtight containers in a refrigerator below 4 °C. The percentage yield of the ethanolic extracts was found to 7.2% (w/w).

Determination of total phenol and flavonoid contents

The total phenol and flavonoid contents in the CDEE were determined by standard techniques ie. Folin-Ciocalteau assay,[21] and the aluminium chloride colorimetric assay[22] respectively.

Free radical scavenging assays

Free radical scavenging activity was determined by 3 methods: DPPH assay,[23] ABTS assay[24] and hydrogen peroxide assay.[25]

Experimental animals

Healthy adult male albino rats of Wistar strain weighing 150–200 g were obtained from animal house of Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, India. The animals were maintained at a controlled temperature of 25 ± 2 °C and constant humidity (40–70%) under a 12 h light–dark cycle. They were fed with standard pellet diet and water ad libitum. Animals were acclimatized to their environment for one week prior to experimentation. Experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institutional Animal Ethical Committee (IAEC) of college.

Acute toxicity study

An acute oral toxicity study was conducted according to OECD 423 guidelines.[26] The extract was administered in a single dose by using specially designed mice oral needle. Animals were deprived of food 3 h prior to dosing. Following the period of fasting the animals were weighed and distributed into six groups of three mice in each group. Groups II, III, IV, V and VI were orally administered the dose of 250, 500, 1000, 2000 and 4000 mg/kg body weight of the extract. Group I served as normal control and received only 2% gum acacia (0.2 ml). All extracts were given in 2% gum acacia. After the extract administration, food was withheld for 2 hours. Effect on grooming, hyper activity, sedation, respiratory arrest, convulsions, motor activity and mortality were observed after 24, 36, 48 and 72 hours.

Experimental design

The experimental protocol was based on a previously reported study.[6–8] CDEE (200, 400 mg/kg body weight) and the standard hepatoprotective drug silymarin were

prepared in 0.5% (w/v) sodium carboxy methylcellulose (CMC). Rats were divided into five groups of 6 animals each. Group I served as a normal control and received 0.5% CMC. Group II served as toxicant control and received 1 ml of 0.5% CMC. Group III received the silymarin standard at a dose of 100 mg/kg body weight. Groups IV and V received CDEE at a dose of 200 and 400 mg/kg body weight, respectively. All the drugs were administered orally using oral feeding needle for 7 days. On the eighth day, all groups received 1 ml/kg body weight of CCl_4, intraperitoneally, expect group I. The blood was collected on the ninth day from the rats by retro-orbital puncture under mild anesthetic (diethyl ether) conditions. The blood was allowed to stand for 30 min at room temperature and then centrifuged to separate the serum. The liver was quickly removed and perfused immediately with ice-cold saline (0.9% sodium chloride). Liver tissue (100 mg) was added to 500 ml of 50 mM Tris buffer (pH 7.4) containing 1 mM EDTA and 10 µg/mL leupeptin and was homogenized using an Elvenjan homogenizer fitted with teflon plunger. The homogenate was centrifuged at 10,000 rpm at 4 °C for 10 min and clear supernatant was used for the estimation of antioxidant parameters (SOD, CAT, MDA, and GSH).

Estimation of biochemical parameters

The separated serum was estimated for the biochemical parameters aspartate aminotransferase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP) by Ecoline diagnostic kits (E–Merck Ltd., Mumbai). Glutathione (GSH) was measured according to the method described by Moron *et al*.[27] Malondialdehyde (MDA) was determined according to the method described by Ohkawa *et al*.[28] Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich[29] and catalase (CAT) according to method described by Aebi.[30]

Histopathological studies

A portion of the liver was cut into two to three pieces of approximately 6 mm^3 size and fixed in phosphate buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5 µm thickness of liver tissue were cut and stained with haematoxylin–eosin. The thin sections of liver were made into permanent slides and examined under high-resolution microscope and photomicrographs were taken.

Statistical analysis

The results of the free radical scavenging assay was expressed as mean ± SEM. The IC_{50} values were calculated by regression analysis. Statistical differences at $P \leq 0.05$ between the groups were analyzed by one-way ANOVA followed by Studentized range multiple comparison test.

RESULTS AND DISCUSSION

Total phenol and flavonoid contents

Total phenol content was estimated at 72.7 ± 2.4 mg gallic acid equivalents/g dry weight extract. Total flavonoid content was 14.3 ± 0.4 mg quercetin equivalent/g dry weight of extract.

Free radical scavenging assays

The antioxidant activity of CDEE was determined by its capacity to scavenge the stable free radicals DPPH, ABTS and hydrogen peroxide which have been widely used to test free radical scavenging activity. The results were compared with the scavenging ability of control samples of standard [Ascorbic acid, Butylated hydroxy anisole (BHA)]. In the DPPH radical scavenging assay, CDEE showed moderate DPPH radical scavenging activity. In this assay, the IC_{50} (the concentration required to scavenge 50% of radical) values of CDEE, ascorbic acid and BHA were 121.3 ± 2.33 µg/ml, 7.3 ± 0.46 and 13.73 ± 1.62 µg/ml, respectively (Table 1). In the ABTS assay, CDEE was found to be very effective scavengers against ABTS radicals and the IC_{50} values of CDEE and BHA were 19.26 ± 1.69 µg/ml and 4.81 ± 0.37 µg/ml, respectively (Table 1). DPPH and ABTS radical scavenging methods are common spectrophotometric procedures for determining antioxidant capacities of components.[31] DPPH radical involves a hydrogen atom transfer process,

Table 1: Free radical scavenging activity of ethanolic extract from *Corchorus depressus*

Sample	Total phenol content	Total flavonoid content	IC_{50} Values		
			DPPH (µg/ml)	ABTS (µg/ml)	H_2O_2 (µg/ml)
CDEE	72.7 ± 2.4[a]	14.3 ± 0.4[b]	121.3 ± 2.33	19.26 ± 1.69	251.93 ± 9.12
Ascorbic acid	–	–	7.3 ± 0.46	4.81 ± 0.37	—
BHA	–	–	13.73 ± 1.62	—	20.10 ± 1.98

[a]Data expressed in mg gallic acid equivalents/g dry weight extract.
[b]Data expressed mg quercetin equivalent/g dry weight of extract.
IC_{50} value was determined to be the effective concentrations at which radicals were inhibited by 50%. Each value was presented as the mean ± S.E.M. of three replicate measurements.

and ABTS radical involves an electron transfer process.[32] The antioxidant activity of *C. depressus* on DPPH and ABTS radicals may be attributed to a direct role in trapping free radicals by donating hydrogen atom or electron. In hydrogen peroxide scavenging assay, the IC_{50} values of CDEE and BHA were 251.93 ± 9.12 μg/ml and 20.10 ± 1.98 μg/ml respectively (Table 1). Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell.[25] Therefore, it is important to discover some chemicals with good scavenging capacity on these reactive oxygen species. Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water hydrogen peroxide. *C. depressus* showed good free radical scavenging activity, indicating the chemicals in this plant performed as good electron donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products. Results from *in vitro* free radical scavenging activity gave us the first evidenceconcerning the mechanism of hepatoprotection of the CDEE and *in vivo* experiments focusing on their scavenging activity were under taken.

Acute toxicity studies

The mice did not show any signs of toxicity or change in general behavior or other physiological activities. No mortality was recorded in animals that were orally administered doses up to 4000 mg/kg of CDEE.

Effect of CDEE on hepatic markers

The results of hepatoprotective effect of CDEE on CCl_4-intoxicated rats were showed in Table 2. Rats treated with CCl_4 developed significant ($P \leq 0.05$) liver damage as by elevated levels of the hepatospecific enzymes AST, ALT and ALP in the serum as compared to control group. A marked elevation in TB level was observed in the group treated with CCl_4 only and it was significantly ($P \leq 0.05$) elevated when compared with the normal values. The TP levels decreased considerably ($P \leq 0.05$) in the toxic group. The groups (IV, V) received the pre-treatment of CDEE at dose levels of 200 and 400 mg/kg body weight significantly ($P \leq 0.05$) prevented the elevated levels of AST, ALT, ALP and TB compare to toxic group. The level of TP was significantly ($P \leq 0.05$) increased in CDEE treaded grouped compare to toxic group. This protective effect of CDEE was dose-dependent. Although silymarin and higher dosages of CDEE showed a strong hepatoprotective effect against CCl_4-induced liver injury, the levels of liver-related biochemical parameters were still significantly higher than those of the control group which did not receive CCl_4 ($P \leq 0.05$). Elevated levels of the serum enzymes, ALT and AST are indicative of cellular leakage and loss of functional integrity of cell membrane in liver.[33] Treatment with CDEE decreased the serum levels of ALT and AST towards their respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. On the other hand ALP is an indicator of pathological alteration in biliary.[34] CCl_4 induced elevation of serum ALP is in line with high levels of serum bilirubin. Effective control of ALP and bilirubin levels in CDEE treatment groups points towards an early improvement in the secretary mechanism of hepatocytes. In this study, CCl_4 also expectedly reduced serum total protein. The TP levels will be depressed in hepatotoxic conditions due to defective protein biosynthesis in liver. The CCl_4 intoxication causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein.[35] The pre-treatment of FSEE well restored the proteins synthesis by protecting the polyribosomes. These biochemical findings were

Table 2: Effect of ethanolic extract from *Corchorus depressus* on biochemical parameters of CCl_4 damaged livers in rats

Design of treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TB (mg/dl)	TP (mg/dl)
Group I Normal control	80.41 ± 3.40	48.98 ± 1.96	157.63 ± 3.79	0.28 ± 0.01	7.06 ± 0.20
Group II CCl_4 control (1.0 ml/kg, i.p.)	291.48 ± 6.71[a]	198.03 ± 3.12[a]	355.30 ± 10.07[a]	0.82 ± 0.03[a]	4.65 ± 0.16[a]
Group III Silymarin (100 mg/kg, p.o)	120.08 ± 3.72[b]	95.48 ± 2.83[b]	226.92 ± 8.85[b]	0.55 ± 0.03[b]	6.56 ± 0.29[b]
Group IV CDEE-200 (200 mg/kg, p.o)	241.09 ± 9.42[b]	165.45 ± 4.78[b]	294.60 ± 6.43[b]	0.73 ± 0.02[b]	5.00 ± 0.15
Group V CDDE-400 (400 mg/kg, p.o)	188.33 ± 6.77[b]	150.16 ± 3.24[b]	248.25 ± 5.68[b]	0.63 ± 0.03[b]	5.42 ± 0.18[b]

Values are mean ± S.E.M., n = 6 animals in each group. Symbols represent statistical significance. CDEE, Ethanolic extract from *Corchorus depressus*; AST, aspartate aminotransferase; ALT, alanine aminotransaminase; ALP, alkaline phosphatase; TB, total bilirubin; TP, total protein.

[a]Activities of AST, ALT, ALP and TB are increased significantly and TP decreased significantly in Group II (CCl_4 control) than Group I (normal control) ($P \leq 0.05$).

[b]Activities of AST, ALT, ALP and TB are inhibited significantly and TP increased significantly in Group IV, V and VI than Group II (CCl_4 control) ($P \leq 0.05$).

Table 3: Effect of ethanolic extract from *Corchorus depressus* on hepatic antioxidant enzymes activity in liver homogenate in damaged liver rats induced by CCl_4

Design of treatment	SOD (U/mg protein)	CAT (U/mg protein)	MDA (U/mg protein)	GSH (U/mg protein)
Group I Normal control	12.52 ± 0.60	28.05 ± 0.95	2.16 ± 0.08	12.40 ± 0.41
Group II CCl_4 control (1.0 ml/kg, i.p.)	4.59 ± 0.41[a]	13.26 ± 0.69[a]	4.08 ± 0.15[a]	7.79 ± 0.38[a]
Group III Silymarin (100 mg/kg, p.o)	10.71 ± 0.37[b]	22.44 ± 0.31[b]	2.80 ± 0.14[b]	10.71 ± 0.23[b]
Group IV CDEE-200 (200 mg/kg, p.o)	6.44 ± 0.24[b]	15.13 ± 0.23	3.38 ± 0.14[b]	8.85 ± 0.14
Group V CDEE-400 (400 mg/kg, p.o)	8.25 ± 0.30[b]	18.22 ± 0.50[b]	3.20 ± 0.20[b]	9.37 ± 0.30[b]

Values are mean ± S.E.M., n = 6 animals in each group. Symbols represent statistical significance. CDEE, Ethanolic extract from *Corchorus depressus*; SOD, superoxide dismutase; CAT, catalase; MDA, monodialdehyde; GSH, glutathione.
[a]Activities of SOD, CAT and GSH are decreased significantly and MDA increased significantly in Group II (CCl_4 control) than Group I (normal control) ($P \leq 0.05$).
[b]Activities of SOD, CAT and GSH are increased significantly and MDA inhibited significantly in Group IV, V and VI than Group II (CCl_4 control) ($P \leq 0.05$).

further substantiated by histopathological studies. The data from this study outlined that the CDEE with doses higher than 200 mg/kg prevented CCl_4-induced liver damage.

Effect of CDEE on hepatic anti-oxidant markers

Results cited in Table 3 clearly reveal significantly ($p \leq 0.05$) decreased level of activity of SOD, CAT, GSH and increased level of activity of MDA in Group II (CCl_4 control) compared to the Group I (normal control). Activity of the antioxidant enzymes, GSH, SOD and CAT were significantly ($p \leq 0.05$) increased in CDEE treated group (Group V) as compared to control group. Treatment with CDEE significantly prevented the rise in MDA levels. CDEE (400 mg/kg) demonstrated maximum hepatoprotection. These results indicate that CDEE protect liver from CCl_4 damage through the scavenging of free radicals. It has been reported that SOD, CAT and GSH constitute a mutually supportive defense against ROS.[3] CCl_4 produces an experimental damage that begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. The toxic metabolite CCl_3 radical is produced which is further converted to trichloromethyl peroxy radical by cytochrome P450 2E1 enzyme.[10] This radical binds covalently to the macromolecules and causes peroxidative degradation of cellular membrane leading to the necrosis of hepatocytes.[36] CCl_4 induced a significant decrease in the levels of activity of the antioxidant enzymes SOD and CAT probably due to protein inactivation by ROS.[37,38] In fact, a decrease of hepatic GSH activity in CCl_4 treated rodents has been reported before.[39] An increase in MDA levels, as evident in our study, suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals.[40] The CDEE was able to partially prevent CCl_4 induced decay of antioxidant enzyme activities; this preventive effect could also be observed at the histological level (Figure 1). It can be said that *C. depressus* contains free radical scavengers that effectively protect the liver against oxidative damage induced by CCl_4, avoiding the oxidation of detoxifying enzymes like CAT and SOD. *C. depressus* could be protective against similar effects by other hepatotoxic chemicals capable of inducing free radicals.

Histopathological studies

Histopathological observations of liver sections from the control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein (Figure 1A). In contrast, the CCl_4 group exhibited the most severe damage of any of the groups. The liver sections in this group showed massive fatty changes, necrosis, ballooning degeneration, broad infiltration of lymphocytes, and the loss of cellular boundaries (Figure 1B). Figure 1C presents the liver section treated with silymarin and CCl_4. The liver sections of the rats treated with CDEE (400 mg/kg body, Figure 1D) showed a relatively normal lobular pattern with a mild degree of fatty change, necrosis, and lymphocyte infiltration.

CONCLUSION

Our findings support the reported therapeutic use of this plant as a hepatoprotective agent in Indian systems of medicine. Several antioxidants have been shown to protect the liver against hepatotoxicants. In this investigation, CDEE showed strong activity on scavenging radicals and potent hepatoprotective effect. However, further investigation should be carried out on the extract to identify the active constituents responsible for hepatoprotection.

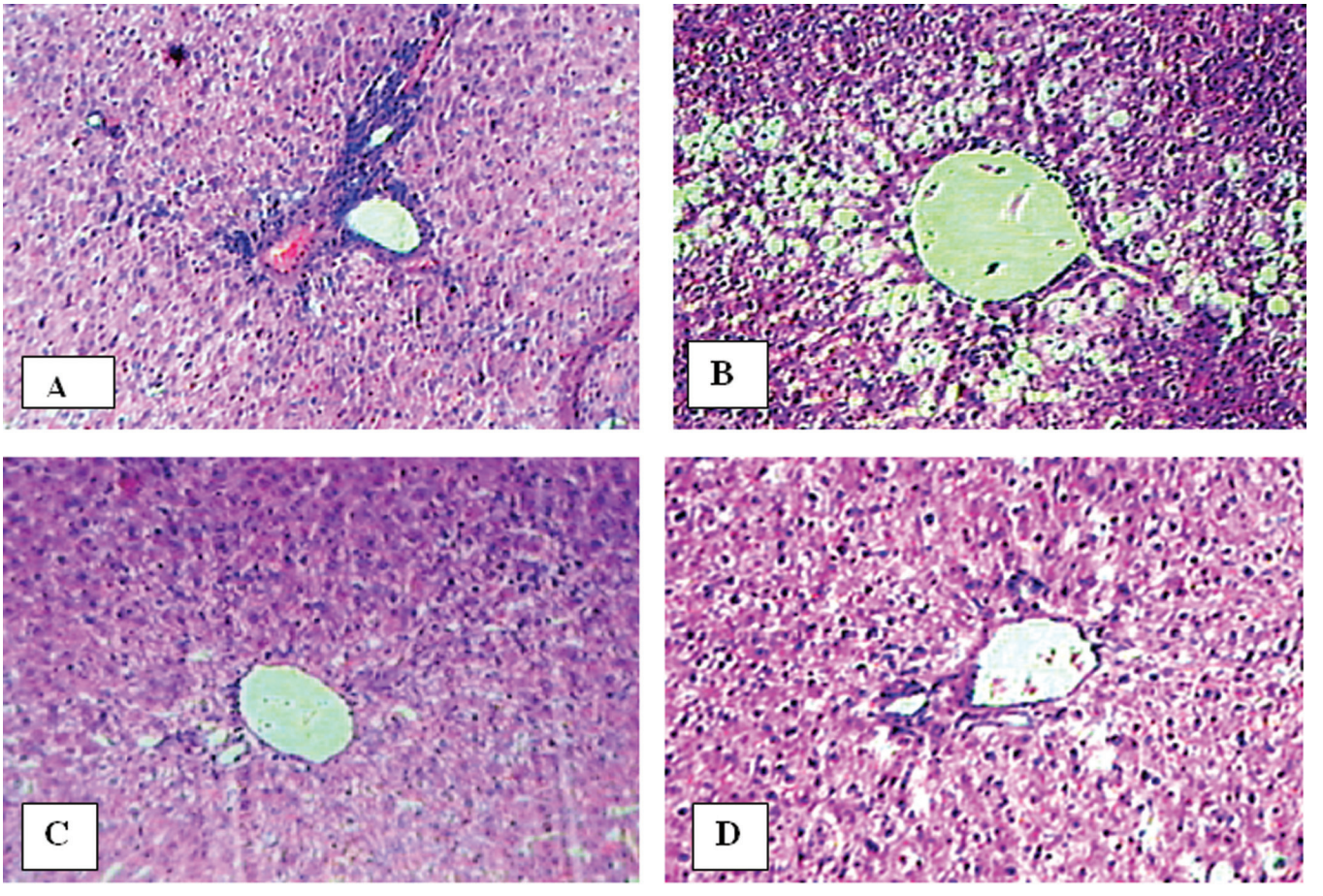

Figure 1. Effect of CDEE (400 mg/kg) and silymarin (100 mg/kg) on liver histopathology of CCl_4 treated male wistar rats. Stain: haematoxylin–eosin, magnification: 100× (A) Vehicle control group section showing normal liver architecture; (B) CCl_4 control section showing ballooning degeneration, massive fatty changes, patches of liver cell necrosis with inflammatory collections; (C) Silymarin + CCl_4 treated group section (Almost near normal); (D) CDEE + CCl_4 treated group section shows lesser damage of hepatocytes and low index of necrosis.

REFERENCES

1. Kong FL, Zhang MW, Kuang RB, Yu SJ, Chi JW, Wei ZC. Antioxidant activities of different fractions of polysaccharide purified from pulp tissue of litchi (*Litchi chinesnsis Sonn*). Carbohydrate Polymers. 2010; 81:612–16.
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. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry & Cell Biology. 2007; 39:44–84.
4. Fu W, Chen JL, Cai YL, Lei YF, Chen LM, Pei L, Zhou DN, Liang XF, Ruan JL. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch. et Sav.) Ching. Journal of Ethnopharmacology. 2010; 130:521–8.
5. Baranisrinivasan P, Elumalai EK, Sivakumar C, Viviyan TS, David E. Hepatoprotective effect of *Enicostemma littorale blume* and *Eclipta alba* during ethanol induced oxidative stress in albino rats. International Journal of Pharmacology. 2009; 5:268–72.
6. 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. Journal of Ethnopharmacology. 2010; 127:777–80.
7. Gomes A, Alama MA, Dattaa P, Bhattacharyab S, Gomes A. Hepatoprotective activity of the edible snail (*Bellamia bengalensis*) flesh extract in carbon tetrachloride induced hepatotoxicity in rats. Journal of Ethnopharmacology. 2011; 38:228–32.
8. Venkatesh P, Dinakar A, Senthikumar N. Hepatoprotective activity of an ethanolic extract of stems of *Anisochilus carnosus* against carbon tetrachloride induced hepatotoxicity in rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3:243–5.
9. Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Slomka M, Madro A, Celinski K, et al. Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. Journal of hepato-biliary-pancreatic surgery. 2003; 10:309–15.
10. Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanism of carbon tetrachloride toxicity. Pharmacology & Therapeutics. 1989; 43:139–54.
11. Mathur M, Sundaramoorthy S. Distribution pattern and growth assessment of *Corchorus depressus* in semi arid Indian desert. Journal of Tropical Ecology. 2008; 49:69–71.
12. Chopra RN, SL Nayar, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi; 1956.
13. Jain A, Katewa SS, Choudhary BL, Galav P. Folk herbal medicines used in birth control and sexual diseases by tribals of southern Rajasthan, India Journal of Ethnopharmacology. 2004; 90:171–7.
14. Kirtikar KR, Basu BD. Indian Medicinal Plants, third ed. Indological and Orient Publishers, New Delhi; 2000.
15. Vohora SB, Shamsi MA, Khan MSY. Antipyretic and analgesic studies on the diacetate of a new triterpenic acid isolated from *Corchorus depressus* L. Journal of Ethnopharmacology. 1981; 2:223–38.
16. Ikram M, Khattak SG, Gilani SN. Antipyretic studies on some indigenous Pakistani medicinal plants: II. Journal of Ethnopharmacology. 1987; 2:185–92.

17. Ali M, Ansari SH. Studies of some herbal drugs used as hair tonic. Hamdard-Medicus. 1998; 41:107–8.
18. Ahmad VU, Ali A, Ali Z, Zafar FN, Zahid M. Two new cycloartane triterpene glucoside from *Corchorus depressus*. Phytochemistry. 1998; 49:829–35.
19. Ahmad VU, Ali A, Ali Z, Zafar FN, Zahid M. Novel cycloartane saponins from *Corchorus depressus* L. Chemical and Pharmaceutical Bulletin. 2000; 48:1591–601.
20. Zahid M, Ali A, Ishurd O, Ahmed A, Ali Z, Ahmad VU. New cycloartane and flavonol glycosides from *Corchorus depressus*. Helvetica Chimica Acta. 2002; 85:689–97.
21. Vernon LS, Orthofer R, Raventos LRM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. 1999; 299:152–78.
22. Chouhan HS, Singh SK. EBA Phytochemical analysis, antioxidant and anti-inflammatory activities of *Phyllanthus simplex*. Journal of Ethnopharmacology. 2011; 137:1337–44.
23. Zimmer AR, Leonardi B, Miron B, Schapoval E, Oliveira JRD, Gosmann G. Antioxidant and anti-inflammatory properties of *Capsicum baccatum*: From traditional use to scientific approach. Journal of Ethnopharmacology. 2012; 139:228–33.
24. Katalinica DV, Modunb TI, Musicb MB. Gender differences in antioxidant capacity of rat tissues determined by 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. Comparative Biochemistry and Physiology, Part C. 2005; 140:47–52.
25. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10:1003–8.
26. OECD (2002). Acute oral toxicity. Acute and toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organization for economical co-operation and development, Paris, June.
27. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta. 1979; 582: 67–78.
28. Misra HP, Fridovich I. Role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 1972; 247:3170–3.
29. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979; 95:351–8.
30. Aebi H. Catalase *in vitro*. Methods in Enzymology. 1984; 105:121–6.
31. Awika JM, Rooney LW, Wu XL, Prior RL, Cisneros-Zevallos L. Screening methods to measure antioxidant activity of Sorghum (Sorghum bicolor) and Sorghum product. Journal of Agricultural and Food Chemistry. 2003; 51;6657–62.
32. Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI. *In vitro* studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. Food Chemistry. 2007; 103;31–7.
33. Drotman RB, Lawhorn GT. Serum enzymes are indicators of chemical induced liver damage. Drug and Chemical Toxicology. 1978; 1:163–71.
34. Ploa GL, Hewitt WR. In: Wallace Hyes, A. (Ed.), Principle and Methods of Toxicology, vol. II. Raven Press, New York 1989; 399.
35. Abidemi Akindele AJ, Ezenwanebea KO, Anunobi CC, Adeyemi OO. Hepatoprotective and in vivo antioxidant effects of Byrsocarpus coccineus Schum. and Thonn. (Connaraceae). Journal of Ethnopharmacology. 2010; 129:46–52.
36. Brattin WJ, Glende Jr., EA, Recknagel RO. Pathological mechanisms in carbon tetrachloride hepatotoxicity. Free Radical Biology and Medicine. 1985; 1:27–38.
37. Hodgson EK, Fridovich I. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: inactivation of the enzyme. Biochemistry. 1975; 14:5294–9.
38. Kono Y, Fridovich I. Superoxide radical inhibits catalase. Journal of Biological Chemistry. 1982; 257:5751–4.
39. Aniya Y, Anders MW. Alteration of hepatic glutathione-S-transferase and release into serum after treatment with bromobenzene and carbon tetrachloride or N-nitrosodimethylamine. Biochemical Pharmacology. 1985; 39:4239–44.
40. Souza MF, Rao VSN, Silveira ER. Inhibition of lipid peroxidation by ternatin, a tetramethoxyflavone from *Egletes viscosa* L. journal of Phytomedicine. 1997; 4:25–9.