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Full Length Article

Preventive and curative effects of *Cocculus hirsutus* (Linn.) Diels leaves extract on CCl4 provoked hepatic injury in rats



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# a b s t r a c t

The present study was designed to estimate the protective or curative potency of an extract from *Cocculus hirsutus* leaves against CCl4 intoxication *via* its antioxidant property in rats. Liver enzyme markers (SGOT, SGPT, ALP, LDH, and bilirubin) and oxidative stress markers {lipid peroxidation (LPO), enzymatic antiox- idants [superoxide dismutase, catalase and glutathione peroxidase] and non-enzymatic antioxidants [re- duced glutathione, vitamin C and E]} were analyzed by spectrophotometry. Histopathological studies on hepatic tissue were also performed by the method of Hematoxylin and Eosin staining. Rats administrated with 30% CCl4 in olive oil intraperitoneally resulted in significant increase in the levels of SGOT, SGPT, ALP, LDH, and bilirubin compared to control rats. Significant elevation of hepatic LPO and depletion of

enzymatic and non-enzymatic antioxidants levels were observed in CCl4 induced rats. When CCl4 induced rats were co-treated with *C. hirsutus* at doses of 250 and 500 mg/kg b·wt, all the altered levels of liver marker enzymes and oxidative stress markers were restored to near control values. Histopathological

studies provided direct evidence of the hepatoprotective effect of *C. hirsutus*. In conclusion, extract from

*C. hirsutus* could protect the liver from CCl4-induced oxidative damage, by scavenging the free radicals generated during the metabolism of CCl4.

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1. Introduction

Hepatic-organ is the imperative organ, which regulates a wide range of physiological processes in the body and it is one of the major organs prone to the oxidative damage by the attack of reac- tive oxygen species [[1,2]](#_bookmark19). Carbon tetrachloride (CCl4) exerted hep- atocellular damage has been largely evaluated and employed model for detecting the novel hepato-protective therapeutics. As the reactive oxygen species are the major cause for the deleterious effects in hepatic disorders, various plant extracts were reported for their hepato-protective activities through their antioxidant activities [[3,4]](#_bookmark20). Natural antioxidants are especially considered as robust candidates to confer protection against chemical induced toxicity [[5,6]](#_bookmark26).

Since the emergence of civilization on the earth, herbal formu- lations have been used to maintain human health and to treat

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various diseases by the vast majority of the World’s population [[7–9]](#_bookmark8). Many traditional systems of medicine in India use a number of medicinal plants and their formulations to cure hepatic disor- ders [[10]](#_bookmark8). In addition to these known plants, there are many other plants used by tribal and folk practitioners which are a potent source of effective hepatoprotective agents that remained unexplored.

Antioxidant property has been reported to play a crucial role in the hepatoprotective capacity of many plants such as *Spirulina maxima*, *Bauhinia hookeri*, and various medicinal herbs [[8,11,12]](#_bookmark8). Treating liver disorders with plant drugs has been an age old tradi- tion by Ayurveda, an indigenous system of medicine in India. Thus the search for potent natural source has become a prime focus for new drug development in pharmaceutical industry for hepatoprotection.

*Cocculus hirsutus* (Linn.) Diels (Menispermaceae), described in ayurvedic literature as patalagarudi is a straggling shrub, widely distributed all over India, especially in dry regions. The leaves and roots of this plant are largely employed in the Indian traditional medicine for a variety of diseases including, hepatic

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obstruction, jaundice, bronchitis, diabetes mellitus, anorexia, gon- orrhoea, and leprosy [[13]](#_bookmark8). *C. hirsutus* is well documented for its anti-inflammatory, analgesic [[14]](#_bookmark8), antidiabetic and spermatogenic

[[15]](#_bookmark9) activities. Considering its varied biological activities and tradi- tional therapeutic use for hepatic disorders, the present study was aimed to evaluate the hepato-protective potential of this plant via its antioxidant property against the deleterious effects of CCl4 induced oxidative damage.

1. Materials and methods
   1. *Chemicals*

2,4-dinitro phenyl hydrazine (DNPH), Disodium phenylphos- phate, Thiobarbituric acid (TBA), 1,1,2,2-tetraethoxy propane (TEP), epinephrine (Adrenaline), Glutathione reductase, reduced glutathione (GSH), Nicotinamide adenine dinucleotide phosphate reduced (NADPH) and cumene hydroperoxide were purchased from Sigma chemical company, USA. All the other chemicals uti- lized for this study were of analytical grade.

* 1. *Plant material collection and extract preparation*

Leaves from *Cocculus hirsutus* plant were collected in and around Tirumala Tirupathi hills, India and compared with the vou- cher specimen deposited at the Department of Botany, Sri Venka- teswara University, Tirupati, India for its identification. The leaves were shade dried for a week and finely powdered using a blender. Ethanol extract of *C. hirsutus* (EECH) was prepared by soxhlation process. The plant powder (100 g) was extracted with 500 mL of 70% ethanol for more than 6 h and concentrated by rotary evaporation and vacuum drying. The yield of the plant

extract was recorded (5.3%) and stored at —20 °C for further use.

* 1. *Animals*

Male Wistar rats (200 ± 50 g; Sri Raghavendra animal suppliers, Bangalore, India) were maintained under standard hygienic condi- tions at 25–28 °C with 12 h light/dark cycle and provided standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libi- tum*. The animal experiments in the present study were conducted

by following the Institute Animal Ethics Committee regulations; approved by the Committee for the Purpose of Control and Super- vision of Experiments on Animals, Government of India.

* 1. *Acute oral toxicity study*

This experiment was performed according to the Organization for Economic Cooperation Development (OECD) guidelines. Ani- mals were divided into one control group and five plant extract treated groups (N = 6). After an overnight fast, rats were dosed orally with ethanol extract of *Cocculus hirsutus* (EECH) in distilled water at doses of 100, 250, 500, 1000, and 2000 mg/kg b. wt. Hourly observations of the animals for any signs of behavioral changes, toxicity and mortality was recorded over a period of 72 h [[16]](#_bookmark10).

* 1. *Experimental design to access the hepato-protective activity of EECH against CCl4 induced liver injury*

Male albino rats were allocated into 4 groups each containing 6 rats. Group I received olive oil only (1 mL/kg body weight, i.p.) as a control, Group II (CCl4 induced) received mL/kg bodyweight of 30% CCl4 in olive oil, i.p. The EECH Extract (250 and 500 mg/kg body weight) was admintrated (orally) once in a day and CCl4 was

administered after every 72 h. Treatment period was 10 days [[4]](#_bookmark25). Blood was collected from all the animals through retro-orbital plexus. After collecting the blood, the rats were sacrificed and their livers were excise, rinsed in ice cold normal saline followed by

0.15 M Tris-HCl (pH 7.4), blotted dry and then frozen at —80 °C

for further biochemical analyses.

* 1. *Estimation of enzyme levels in serum*

Serum was separated from the blood samples by allowing the blood to clot at room temperature for 45 min followed by centrifu- gation (2500 rpm at 30 °C for15 min). Serum transaminases [gluta- mate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT)], alkaline phosphatase (ALP) and lactate dehy- drogenase (LDH) was estimated according to standard protocol described previously [[17–19]](#_bookmark11).

* 1. *Estimation of bilirubin in serum*

Bilirubin content was estimated according to standard protocol by Jain et al. [[20]](#_bookmark12).

* 1. *Preparation of liver homogenate*

Liver homogenates (10%) were prepared by homogenizing the

liver samples in 50 mM phosphate buffer (pH 7.0) and centrifuging the homogenates at 4 °C; 10,000×*g* for 15 min. The supernatant was collected and preserved at —20 °C for further Biochemical ana-

lytical purpose.

All the Biochemical parameters were expressed in the units of activity per mg protein. The protein content in each liver homoge- nate was estimated according to the standard protocol [[21]](#_bookmark13).

* 1. *Estimation of lipid peroxides*

LPO in the liver was determined by the method described ear- lier [[4]](#_bookmark25) by measuring the amount of malondialdehyde (MDA).

* 1. *Quantitative analysis of enzymatic antioxidants*

The mean activities of superoxide dismutase SOD (units/min/ mg protein) [[22,23]](#_bookmark14), catalase (CAT; lmol of H2O2 consumed/min/ mg protein) [[24]](#_bookmark15) and glutathione peroxidase (GPx; lmol of glu- tathione oxidized/min/mg protein) was evaluated by the previ- ously described standard methods [[25]](#_bookmark16).

* 1. *Quantitative analysis of non-enzymatic antioxidants*

Reduced glutathione (GSH) content was determined by its chro- mogenic reaction with dithio-bis-2-nitrobenzoic acid (DTNB) [[26]](#_bookmark17). Vitamin C was estimated by following the standard procedure described by Santhrani et al. [[27]](#_bookmark18). Vitamin E was determined according to the protocol of Ramanathan et al. [[28]](#_bookmark21).

* 1. *Histopathological examination*

Liver tissues were embedded in paraffin blocks and thin sec- tioning was performed according to paraffin slice techniques. The sections were further mounted on to the microscopic slides and stained with Hematoxylin and Eosin stains [[29]](#_bookmark22). These microscopic slides were observed under the light microscope and photographed.

* 1. *Statistical analysis*

The data was calculated and analyzed by using Statistical Pack- age for Social Sciences (SPSS) software; version 15.0. One-way Analysis of Variance for comparing the difference in the means across the groups was performed. Duncan’s Multiple Range Test (DMRT) is used to identify significantly differing group means. The results presented in Tables are mean and standard error (me an ± SE).

1. Results
   1. *Acute oral toxicity*

No mortality, abnormal signs and behavioral changes were observed in rats administered orally up to the dose of 2000 mg/k

g b·wt. of EECH, which is the maximum recommended dose of

any drug for testing acute toxicity by OECD guidelines [[16]](#_bookmark10).

* 1. *Serum enzyme parameters*

A significant increase in the levels of SGOT, SGPT, ALP and LDH in the CCl4 administered rats (Group II) was observed, when com- pared to control (Group I) rats. However, a significant (p < .05) decrease in the levels of these enzymes was observed in the rats treated with two different doses of *C. hirsutus* extract (250 and 500 mg/kg body weight respectively) than in Group II rats ([Table 1](#_bookmark5)).

* 1. *Serum bilirubin*

A marked increase in serum bilirubin was noticed in CCl4 exposed rats when compared to Control rats. Significant decrease in the levels of bilirubin was perceived in Group III and Group IV (250 and 500 mg *C. hirsutus* extract treated) rats on comparing to control (Group I) rats ([Table 1](#_bookmark5)).

* 1. *Hepatic concentration of TBARS*

A pronounced increase in the mean levels of TBARS was noticed in Group II (CCl4 treated) rats on comparing to control (Group I) rats. Treatment with two doses of *C. hirsutus* ethanol extract to Group III and Group IV rats resulted in a significantly (p < .05) low concentrations of TBARS, apparently by hindering hepatic lipid peroxidation ([Table 2](#_bookmark6)).

* 1. *Hepatic enzymatic and non-enzymatic antioxidants*

Hepatic enzymatic and non-enzymatic antioxidants levels in all the four groups of rats are represented in [Table 2](#_bookmark6). In comparison to control rats, a marked decline in the levels of both enzymatic and non-enzymatic antioxidants was noticed in the rats administered

with CCl4. Treatment with two doses of *C. hirsutus* extract in Group III and Group IV rats alleviated the CCl4 damage by retrieving the declined levels of both enzymatic and non-enzymatic antioxidants to near control values.

* 1. *Histopathological examination*

[Fig.1](#_bookmark7) represents the histopathological examination of hepatic tissue of all the four groups of rats. Extensive damage to the his- toarchitecture of hepatic tissue (the disruption of the lattice nature of the hepatocyte, damaged cell membranes, disintegrated central vein and damaged hepatic sinusoids; [Fig. 1](#_bookmark7)B) of the rats exposed to CCl4 was noticed compared to the histoarchitecture of hepatocytes in control rats ([Fig. 1](#_bookmark7)A). Minimum disturbance of the hepatic cellu- lar structure was noticed in both the *C. hirsutus* extract treated groups ([Fig. 1](#_bookmark7)C and D), when compared to CCl4 alone treated rats.

1. Discussion

Hepatic injuries caused by CCl4 are the specific symptoms of xenobiotic-induced hepatotoxicity and regularly used models for the screening of hepatoprotective drugs [[30]](#_bookmark23). The major causes of CCl4 induced hepatic damage is generation of free radicals causing lipid peroxidation and contaminant decrease in antioxidant enzymes [[30]](#_bookmark23). As free radicals play a vital role in CCl4-induced hep- atotoxicity, it seems reasonable that compounds that counteract such radicals may have hepatoprotective effect. Several natural products have been reported to protect against CCl4-induced hep- atotoxicity [[31–34]](#_bookmark24).

Spotlight of the present study was on investigating the role of *C. hirsutus* against the hepatotoxicity of CCl4 and to understand the possible mode of action in hepatoprotection. Direct evidence of CCl4 causing liver damage was observed by the manifestation of alterations in various hepatic parameters {elevated MDA concen- tration, depleted levels of non-enzymatic antioxidants (GSH, Vita- min C and E), reduced levels of antioxidant enzymes (SOD, CAT, GPx)} in CCl4 alone administered (Group II) rats in comparison to control rats. The increase in levels of SGOT, SGPT, ALP and LDH in Group II rats also suggests the hepatotoxicity in these rats. To elucidate the hepatoprotective activity of ethanol extract of *C. hir- sutus*, this plant extract was administered in the concentrations of 250 and 500 mg/kg body weight to the respective group of rats. This concentration of the extract was chosen on the basis of earlier related studies suggestion that this dose would be effective [[14,35]](#_bookmark8). Serum transaminases, ALP, and LDH are well chosen specific markers of hepatic damage [[36]](#_bookmark27). CCl4 induced damage of hepato- cytes can leads to effect on their transport function and membrane permeability, leading to these enzymes leakage from the cells and releasing them into the blood stream [[37]](#_bookmark27). In the present study, rats induced with CCl4 showed significant elevation of all these enzymes demonstrating serious damage of hepatocytes. Hyper bilirubinaemia which is the indicator of severe hepatic damage

Table 1

Effects of ethanol extract from *Cocculus hirsutus* on serum GOT, GPT, ALP, LDH, and bilirubin levels in control and experimental rats against CCl4 induced toxicity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Group I | Group II | Group III | Group IV | ANOVA F-value |
| SGOT (IU/L) | 90.3 ± 10.3a | 298 ± 8.5b | 185 ± 6.8c | 110 ± 7.3d | 617.01 |
| SGPT (IU/L) | 51.6 ± 12.3a | 321 ± 9.81b | 138 ± 5c | 98.3 ± 2d | 927.22 |
| ALP (IU/L) | 173.6 ± 10.1c | 318 ± 6.7a | 254 ± 5d | 218 ± 6b | 895.25 |
| LDH (IU/L) | 941 ± 14.4d | 2481 ± 20.4a | 1468 ± 11b | 1043 ± 15c | 511.19 |
| Bilirubin (mg/dL) | 1.21 ± 0.03 c | 2.1 ± 0.06a | 1.8 ± 0.05d | 1.4 ± 0.05b | 38.77 |

Group I: (control); Group II: CCl4 treated; Group III: CCl4 + 250 mg/kg EECH; Group IV: CCl4 + 500 mg/kg EECH.

Values are expressed as Mean ± SE of six rats in each group. Means having same superscript in each row do not differ significantly at 0.05 levels by Duncan’s Multiple Range Test (DMRT).

All the enzyme activity units = IU/L: International Units/Liter; mg/dL: milligrams/deciliter.

Table 2

Effect of ethanol extract of *Cocculus hirsutus* on hepatic levels of TBARS, enzymatic and non-enzymatic antioxidants in control and experimental rats against CCl4 induced toxicity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Group I | Group II | Group III | Group IV | ANOVA F-value |
| LPO | 1.28 ± 0.02a | 2.48 ± 0.06b | 1.93 ± 0.04c | 1.53 ± 0.03d | 125.89 |
| SOD | 0.402 ± 0.03d | 0.21 ± 0.01b | 0.31 ± 0.01a | 0.39 ± 0.02c | 101.59 |
| CAT | 64.32 ± 5.63c | 43.12 ± 3.61a | 51.21 ± 2.61b | 59.31 ± 1.32d | 339.73 |
| GPx | 53.46 ± 3.43d | 36.46 ± 1.21c | 39.86 ± 1.05b | 44.32 ± 1.25a | 495.52 |
| GSH | 5.48 ± 0.69a | 2.43 ± 0.11b | 3.61 ± 0.06c | 4.18 ± 0.01d | 398.41 |
| Vitamin C | 3.19 ± 0.06c | 1.54 ± 0.16d | 2.01 ± 0.13b | 2.94 ± 0.03a | 580.66 |
| Vitamin E | 1.53 ± 0.02a | 0.71 ± 0.01b | 0.89 ± 0.03c | 1.19 ± 0.04d | 179.72 |

Group I: (control); Group II: CCl4 treated; Group III: CCl4 + 250 mg/kg EECH; Group IV: CCl4 + 500 mg/kg EECH.

Values expressed are Mean ± SE of six rats in each group. Means having same superscript in each row do not differ significantly at 0.05 level by Duncan’s Multiple Range Test (DMRT). Lipid peroxidation (LPO) – nano moles of MDA produced/mg protein; CAT–mmoles of H2O2 utilized/min/mg protein; SOD–units/min/mg protein; GPX – mmoles of GSH oxidized/min/mg protein; GSH – mg of reduced glutathione/mg protein; Vitamin C – mg/mg protein; Vitamin E – mg/mg protein.

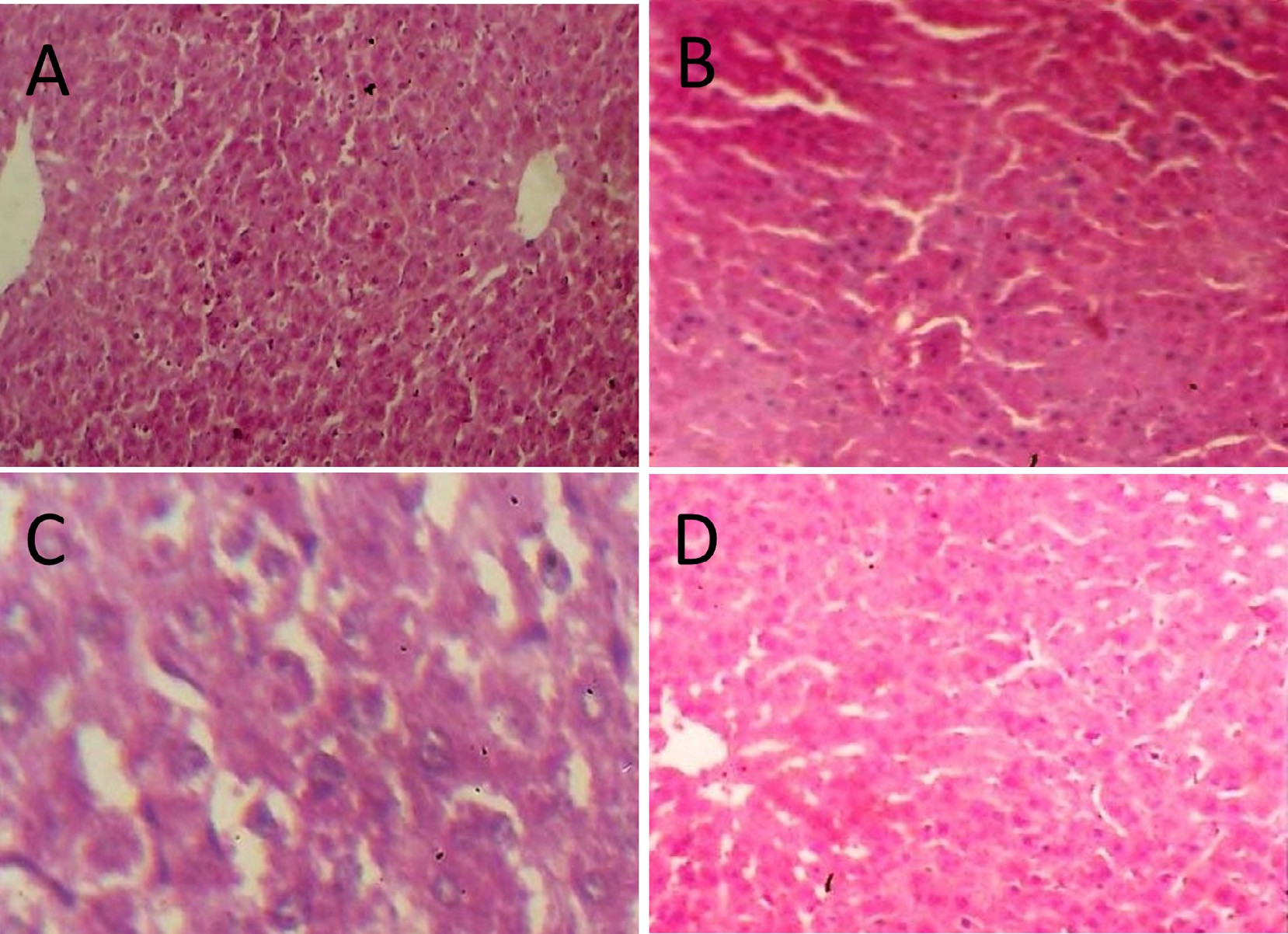


Fig. 1. Light microscopic photographs of liver histology in control and experimental rats. (A) Control mice showing normal histoarchitecture of hepatocytes, (B) A liver section of rat received CCl4 showing hepatocellular damage, (C) A liver section of rat received CCl4 + 250 mg/kg b·wt. EECH showing minimal damage to liver cells, and (D) A liver section of rat received CCl4 + 500 mg/kg b. wt. EECH showing almost normal architecture of liver. Hematoxilin and Eosin stain; Original magnification ×10.

[[38]](#_bookmark27), was noticed in rats treated with CCl4. Treatment with ethanol extract of *C. hirsutus*, restored all the altered levels of liver marker enzymes and bilirubin in a dose dependent manner demonstrating its curative potential to maintain the normal functional status of the hepatic tissue.

The level of TBARS is related to lipid peroxidation and the lipid peroxide levels in liver were found to be considerably elevated in CCl4-challenged rats [[39]](#_bookmark28). These free radicals trigger cell damage through two mechanisms namely covalent binding to cellular macromolecules and lipid peroxidation which affect the ionic per- meability of the membrane preventing the disintegration and sol- ubilization of membrane structure. The reduced TBARS formation after treatment with the EECH may be attributed to the antioxidant activity of the plant by scavenging the CCl3-radical generated due to the metabolic transformation of CCl4 in the liver. The antioxi- dant enzymes SOD, catalase and GPx constitute primary defense mechanisms against reactive oxygen species (ROS).

The antioxidant enzymes SOD, CAT and GPx constitute a mutu- ally supportive group of defense against ROS. The decrease in the levels of these antioxidant enzymes in liver of CCl4-treated rats may be due to the elevated lipid peroxidation or inactivation of the enzymes by cross-linking with malondialdehyde; this will cause massive accumulation of free radicals, which could further stimulate lipid peroxidation [[40]](#_bookmark28). Administration of EECH signifi- cantly increased the activities of SOD, CAT, and GPx in the rats induced with CCl4; this suggests administration of *C. hirsutus* etha- nol extract appears to have brought about a remarkable improve- ment in the activity of these antioxidant enzymes in CCl4 intoxicated rats. Similar improvement of antioxidant enzymes by supplementation of phytochemicals and extracts such as polyphe- nols from *Bauhinia hookeri* [[41]](#_bookmark28) and *Juniperus phoenicea* [[42]](#_bookmark28) extract against CCl4 induced oxidative stress was recently reported.

Glutathione, Vitamin C and E protect cells against CCl4 induced toxicity by preventing the formation of electrophiles, oxidation of

proteins sulfhydryl groups and by scavenging free radicals [[43]](#_bookmark28). The key protective mechanism of glutathione against oxidative stress is acting as a co-factor for several detoxifying enzymes and also regenerate Vitamins C and E back to their active forms [[38]](#_bookmark27). In the present study, rats administered with two doses of EECH leaves had observed significantly higher levels of GSH, vitamin C and E when compared with CCl4 induced group and also near to the values obtained in normal rats. These results suggests that *C.* *hirsutus* extract appears to be a potent hepatoprotective agent against CCl4 induced oxidative damage by maintaining the GSH, vitamin C and E levels. In the present study, replenishment of GSH, Vitamins C and E by the supplementation of plant extract to confer protection against CCl4-induced liver damage, goes in accordance with Lavanya et al. (2012) [[4]](#_bookmark25) and Manubolu et al. (2014) [[22]](#_bookmark14) reports.

To provide direct evidence on protective effect of EECH against CCl4 induced toxicity, histopathological changes in liver tissues were evaluated. Marked disruption of the cellular structure of liver was noted in rats challenged with CCl4. Minimal disruption of the hepatocellular structure was noted in two doses of EECH treated groups of rats; these results provided supportive evidence for bio- chemical analysis (SGOT, SGPT, SALP, and LDH activities and MDA levels approximated to the levels in normal rats).

In conclusion, the results of this study indicated that EECH has a potent hepatoprotective activity on carbon tetrachloride induced hepatocellular destruction in rats. From the present experiments it was elucidated that the hepatoprotective nature of EECH may be due to its antioxidative and free radical scavenging properties. It also reveals that *C. hirsutus* is a robust medicinal herb for devel- oping as a phytomedicine against hepatic disorders and further studies are essential in the direction of isolating the active princi- ple of the plant which acts as an effective antihepatotoxic agent.

Authors’ contribution

GL: Designed the research, conducted the research, analyzed the data, drafted and revised the manuscript. MM: helped with portions of conducting research and revised the manuscript.

PK and PRP: revised the manuscript.

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Conflict of interests

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References

1. [Wu S, Yue Y, Tian H, Li Z, Li X, He W, et al. Carthamus red from *Carthamus*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0005)[*tinctorius* L. exerts antioxidant and hepatoprotective effect against CCl4-](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0005) [induced liver damage in rats via the Nrf2 pathway. J Ethnopharmacol](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0005) [2012;148(2):570–8](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0005).
2. [Li S, Tan H-Y, Wang N, Zhang Z-J, Lao L, Wong C-W, et al. The role of oxidative](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0010) [stress and antioxidants in liver diseases. Int J Mol Sci 2015;16(11):26087–124](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0010).
3. [Cheng N, Ren N, Gao H, Lei X, Zheng J, Cao W. Antioxidant and](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0015) [hepatoprotective effects of *Schisandra chinensis* pollen extract on CCl4-](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0015) [induced acute liver damage in mice. Food Chem Toxicol 2013;55:234–40](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0015).
4. [Lavanya G, Voravuthikunchai SP, Towatana NH. Acetone extract from](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0020) [*Rhodomyrtus tomentosa*: a potent natural antioxidant. Evidence Based](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0020) [Complementary Altern Med 2012;2012:8](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0020).
5. [Lavanya G, Sivajyothi R, Manjunath M, Parthasarathy P. Fate of biomolecules](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0025) [during carbon tetrachloride induced oxidative stress and protective nature of](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0025)

[*Ammannia baccifera* Linn.: a natural antioxidant. Int J Green Pharm 2009;3](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0025) [(4):300–5](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0025).

1. [Manjunath M, Lavanya G, Sivajyothi R, Vijaya Sarathi Reddy O. Antioxidant and](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0030) [radical scavenging activity of *Actiniopteris radiata* (Sw.) link. Asian J Exp Sci](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0030) [2011;25(1):73–80](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0030).
2. [Myagmar BE, Shinno E, Ichiba T, Aniya Y. Antioxidant activity of medicinal herb](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0035) [*Rhodococcum vitis-idaea* on galactosamine-induced liver injury in rats.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0035) [Phytomedicine 2004;11(5):416–23](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0035).
3. [Chaudhary GD, Kamboj P, Singh I, Kalia AN. Herbs as liver savers-a review.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0040) [Indian J Nat Prod Resour 2010;14:397–408](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0040).
4. [Pathakoti K, Goodla L, Manubolu M, Tencomnao T. Metabolic alterations and](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0045) [the protective effect of punicalagin against glutamate-induced oxidative](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0045) [toxicity in HT22 cells. Neurotox Res 2017;31:521–31](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0045).
5. [Zakaria ZA, Rofiee MS, Somchit MN, Zuraini A, Sulaiman MR, Teh LK, et al.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0050) [Hepatoprotective activity of dried- and fermented-processed virgin coconut](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0050) [oil. Evidence Based Complementary Altern Med 2011;2011:8](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0050).
6. [Torres-Duran PV, Miranda-Zamora R, Paredes-Carbajal MC, Mascher D, Ble-](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0055) [Castillo J, Diaz-Zagoya JC, et al. Studies on the preventive effect of *Spirulina*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0055)[*maxima* on fatty liver development induced by carbon tetrachloride, in the rat.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0055) [J Ethnopharmacol 1999;64(2):141–7](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0055).
7. [Al-Sayed E, Martiskainen O, et al. Hepatoprotective and antioxidant effect of](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0060) [*Bauhinia hookeri* extract against carbon tetrachloride-induced hepatotoxicity](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0060) [in mice and characterization of its bioactive compounds by HPLC-PDA-ESI-MS/](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0060) [MS. Biomed Res Int 2014;2014:14. 245171](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0060).
8. [Thakare S, Jain H, Patil S, Upadhyay U. Hepatoprotective effect of *Cocculus*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0065)[*hirsutus* on bile duct ligation-induced liver fibrosis in *Albino wistar* rats.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0065) [Bangladesh J Pharmacol 2009;4:126–30](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0065).
9. [Sarvankumar G, Rajesh K, Sengottuvelu S, Chiranjeev G, Hareesh D. Evaluation](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0070) [of analgesic and anti-inflamatory activity of methanolic extract of *Cocculus*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0070)[*hirsutus* leaves. Int Res J Pharm 2011;2(12):230–4](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0070).
10. [Sangameswaran B, Jayakar B. Anti-diabetic and spermatogenic activity of](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0075)

[*Cocculus hirsutus* (L) Diels. Afr J Biotechnol 2007;16(10):1212–6](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0075).

1. OECD. Guidelines for testing of chemicals. Revised draft guideline 423: Acute oral toxicity; 2000.
2. [Anusuya N, Raju K, Manian S. Hepatoprotective and toxicological assessment](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0085) [of an ethnomedicinal plant *Euphorbia fusiformis* Buch.–Ham.ex D.Don. J](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0085) [Ethnopharmacol 2010;127(2):463–7](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0085).
3. [Sushma N, Devasena T. Aqueous extract of *Trigonella foenum graecum*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0090)[(fenugreek) prevents cypermethrin-induced hepatotoxicity and](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0090) [nephrotoxicity. Hum Exp Toxicol 2010;29(4):311–9](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0090).
4. [Lavanya G, Manjunath M, Sivajyothi R, Parthasarathy PR. Safety evaluation of](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0095) [the ethanol extract of *Ammannia baccifera* (Lythraceae): assessment of acute](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0095) [and subacute toxicity. J Pharm Res 2010;3(11):2634–7](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0095).
5. [Jain A, Soni M, Deb L, Jain A, Rout SP, Gupta VB, et al. Antioxidant and](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0100) [hepatoprotective activity of ethanolic and aqueous extracts of *Momordica*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0100)[*dioica* Roxb. leaves. J Ethnopharmacol 2008;115(1):61–6](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0100).
6. [Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0105) [folin phenol reagent. J Biol Chem 1951;193(1):265–75](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0105).
7. [Manubolu M, Goodla L, Ravilla S, Thanasekaran J, Dutta P, Malmlof K, et al.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0110) [Protective effect of *Actiniopteris radiata* (Sw.) Link. against CCl4](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0110) [induced](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0110) [oxidative stress in albino rats. J Ethnopharmacol 2014;153(3):744–52](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0110).
8. [Tripathi UN, Chandra D. The plant extracts of *Momordica charantia* and](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0115) [*Trigonella foenum-graecum* have anti-oxidant and anti-hyperglycemic](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0115) [properties for cardiac tissue during diabetes mellitus. Oxid Med Cell Longev](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0115) [2009;2(5):290–6](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0115).
9. [Ebaid H, Bashandy SA, Alhazza IM, Rady A, El-Shehry S. Folic acid and](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0120) [melatonin ameliorate carbon tetrachloride-induced hepatic injury, oxidative](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0120) [stress and inflammation in rats. Nutr Metab 2013;10(1):1743–7075](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0120).
10. [Kumar Mishra S, Singh P, Rath SK. Protective effect of quercetin on](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0125) [chloroquine-induced oxidative stress and hepatotoxicity in mice. Malar Res](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0125) [Treat 2013;2013:10](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0125).
11. [Messarah M, Amamra W, Boumendjel A, Barkat L, Bouasla I, Abdennour C, et al.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0130) [Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0130) [damage in rat liver and erythrocytes. Toxicol Ind Health 2012;18:2012](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0130).
12. [Santhrani T, Maheswari E, Saraswathy GR. Amelioration of carbamazepine](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0135) [induced oxidative stress and hematotoxicity by vitamin C. Spatula DD 2012;2](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0135) [(3):173–80](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0135).
13. [Ramanathan S, Kuppusamy A, Nallasamy VM, Perumal P. Antitumor effects](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0140) [and antioxidant role of *Scutia myrtina* in N-Nitroso-diethylamine (NDEA)](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0140) [induced hepatocellular carcinoma in rats. Asian J Pharm Biol Res 2011;1](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0140) [(2):71–8](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0140).
14. [Dhanasekaran JJ, Ganapathy M. Hepatoprotective effect of *Cassia auriculata* L.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0145) [leaf extract on carbon tetrachloride intoxicated liver damage in Wister Albino](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0145) [rats. Asian J Biochem 2011;6(1):104–12](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0145).
15. [Chen S, Zou L, Li L, Wu T. The protective effect of glycyrrhetinic acid on carbon](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0150) [tetrachloride-induced chronic liver fibrosis in mice via upregulation of Nrf2.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0150) [PLoS One 2013;8(1):14](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0150).
16. [Huo HZ, Wang B, Liang YK, Bao YY, Gu Y. Hepatoprotective and antioxidant](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0155) [effects of licorice extract against CCl4-induced oxidative damage in rats. Int J](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0155) [Mol Sci 2011;12(10):6529–43](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0155).
17. [Rudnicki M, Silveira MM, Pereira TV, Oliveira MR, Reginatto FH, Dal-Pizzol F,](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0160) [et al. Protective effects of *Passiflora alata* extract pretreatment on carbon](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0160) [tetrachloride induced oxidative damage in rats. Food Chem Toxicol 2007;45](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0160) [(4):656–61](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0160).
18. [Bhattacharjee R, Sil PC. Protein isolate from the herb, *Phyllanthus niruri* L.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0165) [(Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0165)

[induced liver damage via its antioxidant properties. Food Chem Toxicol](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0165) [2007;45(5):817–26](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0165).

1. [Fahmy NM, Al-Sayed E, Abdel-Daim MM, Karonen M, Singab AN. Protective](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0170) [effect of *Terminalia muelleri* against carbon tetrachloride-induced hepato and](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0170) [nephro-toxicity in mice and characterization of its bioactive constituents.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0170) [Pharm Biol 2016;54(2):303–13](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0170).
2. [Badole S, Patel N, Bodhankar S, Jain B, Bhardwaj S. Antihyperglycemic activity](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0175) [of aqueous extract of leaves of *Cocculus hirsutus* (L.) Diels in alloxan-induced](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0175) [diabetic mice. Indian J Pharmacol 2006;38(1):49](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0175).
3. [Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0180) [biomarkers of hepatotoxicity. Toxicology 2008;245(3):194–205](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0180).
4. [Jeong SC, Kim SM, Jeong YT, Song CH. Hepatoprotective effect of water extract](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0185) [from *Chrysanthemum indicum* L. flower. Chin Med 2013;8(1):1749–8546](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0185).
5. [Olaleye MT, Akinmoladun AC, Ogunboye AA, Akindahunsi AA. Antioxidant](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0190) [activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0190)[Linn against acetaminophen-induced liver damage in rats. Food Chem Toxicol](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0190) [2010;48(8–9):2200–5](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0190).
6. [Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, et al. Effect of](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0195) [vitamin D deficiency and replacement on endothelial function in](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0195) [asymptomatic subjects. J Clin Endocrinol Metab 2009;94(10):4023–30](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0195).
7. [Hung GD, Li PC, Lee HS, Chang HM, Chien CT, Lee KL. Green tea extract](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0200) [supplementation ameliorates CCl4-induced hepatic oxidative stress, fibrosis,](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0200) [and acute-phase protein expression in rat. J Formos Med Assoc 2012;111](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0200) [(10):550–9](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0200).
8. [Al-Sayed E, Abdel-Daim MM, Kilany OE, Karonen M, Sinkkonen J. Protective](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0205) [role of polyphenols from *Bauhinia hookeri* against carbon tetrachloride-](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0205) [induced hepato- and nephrotoxicity in mice. Ren Fail 2015;37(7):1198–207](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0205).
9. [Laouar A, Klibet F, Bourogaa E, Benamara A, Boumendjel A, Chefrour A, et al.](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0210) [Potential antioxidant properties and hepatoprotective effects of *Juniperus*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0210)[*phoenicea* berries against CCl4](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0210) [induced hepatic damage in rats. Asian Pac J Trop](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0210) [Med 2017;10(3):263–9](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0210).
10. [Veigas JM, Shrivasthava R, Neelwarne B. Efficient amelioration of carbon](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0215) [tetrachloride induced toxicity in isolated rat hepatocytes by *Syzygium cumini*](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0215)[Skeels extract. Toxicol In Vitro 2008;22(6):1440–6](http://refhub.elsevier.com/S2314-808X(17)30266-X/h0215).