

Original Article

Screening of *in vitro* Cytotoxicity, Evaluation of Hepatoprotective and Oxidative Stress Inhibiting Potential of *Clitoria ternata* in Carbon Tetrachloride Induced Hepatic Damage in Rats

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ABSTRACT: **Background:** *Clitoria ternata* is a herb extensively used in treating a wide variety of ailments. Early researches of *C.ternata* mainly focused on the evaluation of antitoxic roles of methanolic extract. **Objective:** The present study focused on evaluating the protective roles of petroleum ether (PE) and chloroform (CE) extracts of *C.ternata* on CCl₄ induced liver damage. **Materials and Methods:** Hepatoprotective activity was determined by assessing the levels of various hepatic markers like SGOT, SGPT, ALP and total bilirubin content. Inhibition of oxidative stress (antioxidant) analysis was done by estimating the levels of SOD, CAT, TBARS and total protein content. In addition, our study also assesses the anticancer activity against three cell lines namely HeLa, K-562 and MCF-7. **Results:** CCl₄ caused a marked rise in serum marker levels. Both PE and CE of *C.ternata* decreased the alterations of these effects in the experimental animals and protected the liver tissues against CCl₄ induced oxidative stress by increasing the antioxidant defense. Furthermore, both the extracts also exhibited anticancer activity by inhibiting cancer cell proliferation. **Conclusion:** The results showed that the *C.ternata* extracts possess cytotoxic, antioxidant, hepatoprotective roles and supports their use in traditional medicine.

KEYWORDS: Liver, *Clitoria ternata*, CCl₄, cytotoxic activity, lipid peroxidation, antioxidants

INTRODUCTION

During the course of aerobic metabolic reactions, considerable amounts of reactive oxygen species (ROS) are generated, which leads to the production of free radicals. These free radicals cause lipid peroxidation, resulting in degradation and inactivation of various important biomolecules.^[1] Free radical damage may lead to cancer.^[2] Antioxidants fight against radicals and may prevent some of the damage by free radicals.^[3] Laboratory evidence from chemical, cell culture and animal studies indicate that antioxidants may slow or possibly prevent the devel-

opment of cancer.^[4] Almost all the organs and systems in the human body encounter stress due to some levels free radicals. Liver is the most important metabolic organ and plays a pivotal role in various physiologic processes including metabolism, secretion and storage.^[5] Liver is also important in managing the internal environment, biochemical conversion of endogenous and exogenous chemicals and others into harmless easily excretable form.^[6] Hence, it is referred as the major site of xenobiotic metabolism. Various agents like drugs, alcohol, viruses and environmental chemicals are capable of causing some degree of liver injury. Liver damage is associated with cellular necrosis, increases in tissue lipid peroxidation and a depletion in the tissue GSH levels.^[7] Conventional drugs used in the treatment of liver ailments are not completely safe and also have unwanted side effects. Therefore, it is necessary to search for potentially active alternative medicinal properties, especially using herbal drugs for the treatment of liver disease for better efficacy and safety to replace currently used drugs.^[8]

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Clitoria ternata Linn belongs to family Fabaceae; commonly known as 'Aparajita' or shankupushpi. It is a perennial climber widely used to treat a wide variety of ailments. *C.ternata* exhibits various properties including anxiolytic, antidepressant, anticonvulsant anti stress, anti-inflammatory, analgesic and antipyretic properties.^[9-12] Chemical constituents like malonylated flavonol glycosides namely kaempferol 3-O- β -glycoside, quercetin 3-O- β -glycoside and myricetin 3-O- β -glycosides^[13] and also eight different anthocyanins were isolated and reported from the flower petals of *C. ternata*.^[14] Hepatoprotective activity of methanolic extracts of leaf, root and flowers was reported earlier.^[15] The leaves have been demonstrated to exhibit *in vitro* antioxidant properties and contain some phytochemical compounds like flavonoids, tannins, alkaloids, terpenes.^[16,17] Therefore, to validate the pharmacognostic properties, the present study was performed to evaluate the cytotoxic, antioxidant and hepatoprotective effects of petroleum ether (PE) and chloroform extracts (CE) of leaves of *C. ternata* against carbon tetrachloride (CCl₄) induced liver damage in albino rats.

MATERIALS AND METHODS

Nitroblue tetrazolium, carbon tetrachloride, Folin-Ciocalteu reagent, and hydrogen peroxide were purchased from Merck (Germany). All other chemicals were analytical grade.

Successive Soxhlet extraction and fractionation of the plant extract

The *C.ternata* leaves were air dried in sun light at 40°C. Leaves were separated and then ground into powder using a grinding apparatus and subsequently extracted in a Soxhlet apparatus by the serial extraction method with solvents based on the increase in polarity using petroleum ether, and chloroform. Solvent elimination under reduced pressure afforded the petroleum ether (2% w/w yield) and chloroform extract (2.4% w/w yield) respectively. The extracts were then kept in desiccators at room temperature (22–24 °C) prior to use in our experiments.

Anticancer activity

Anticancer activity (cytotoxic activity) of PE and CE of *C.ternata* was tested against HeLa, K562 and MCF-7 carcinogenic cell lines.

Cell culture and MTT assay

Three different human cancer cell lines namely HeLa, K-562 and MCF-7, which were derived from cervical cancer cells, myelogenous leukemia and breast cancer cells respectively, were used in this study. These were procured

from National Centre for Cell Science, Pune. All cells were grown in minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in a 5% CO₂ incubator. The MTT assay developed by Mossman was modified and used to determine the inhibitory effects on cell growth *in vitro*.^[18] In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of 5×10³ cells/well in growth medium and cultured at 37°C in 5% CO₂ to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test compounds (12.5, 25, 50,100 and 200 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The plantextract was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 5 µl of fresh MTT (0.5 mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 492 nm on an ELISA reader, Anthos 2020 spectrophotometer. Samples were diluted and corrected so that they were within the dynamic range of the optical density.

In vivo experiments

Wistar albino rats of both the sexes (weighing about 120–175gms) were used in the study were procured from Mahaveer enterprises, Hyderabad and were placed at random and allocated to treatment groups in clean polypropylene cages (38 × 23 × 10 cms) with not more than four animals per cage. Animals were housed and maintained under standard laboratory conditions, at a temperature of 24 ± 2°C and relative humidity of 30 – 70% with dark and light cycles (12/12 hour). The animals were allowed free access to standard pellet diet (Hindustan lever, Kolkata, India) and water *ad libitum* during the course of study. The animals were acclimatized to laboratory conditions for 10 days before commencement of experiment. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC.

Hepatoprotective activity against carbon tetrachloride (CCl₄) induced liver damage in rats

Hepatoprotective activity of PE and CE of *C. ternata* against CCl₄ induced toxicity was carried according to previous method.^[19] Healthy albino rats were divided

into 5 groups each containing 4 animals. Group-I control received liquid paraffin (1 ml/kg body weight, p.o). Group-II toxic control received 30% CCl₄ in liquid paraffin (1 ml/kg body weight, i.p). Group-III received chloroform extract (100 mg/kg p.o) once in a day. Group-IV received petroleum ether extract (100 mg/kg p.o) once in a day. Group-V received standard drug Silymarin (25 mg/kg p.o) once in a day. Treatment duration was a period of 10 days and the dose of CCl₄ was administered after every 72 h for all the treatment groups except group-I. Animals were sacrificed 24 h after the last injection. Blood was collected, allowed to clot and serum separated. The liver was excised and used for biochemical studies.

Biochemical studies

Blood was obtained from all animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including determination of serum bilirubin,^[20] serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT)^[21] and alkaline phosphatase (ALP).^[22]

Antioxidant studies

After collection of blood samples, the rats were sacrificed and their livers excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl (pH7.4) and blotted dry. A 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C and used for the estimation of levels of superoxide dismutase (SOD),^[23] catalase (CAT)^[24] and total protein.^[25]

Determination of thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation in liver tissues was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS).^[26] Briefly, to 0.2 ml of sample, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA were added. The volume of the mixture was made up to 4 ml with distilled water and then heated at 95°C in a water bath for 60 min. After incubation, the tubes were cooled to room temperature and the final volume was made to 5 ml with distilled water in each tube. Then 5 ml of n-butanol: pyridine mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min the upper organic layer was taken and its OD was read at 532 nm against a blank containing reaction mixture except the sample. The levels of lipid peroxides were quantified by

the formation of thiobarbituric acid reactive substances (TBARS) namely malonaldehyde (MDA) and expressed as MDA/100 gram of liver tissue using an extinction coefficient of MDA is $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Histopathological studies

Small pieces of liver tissues in each group were collected in 10% neutral buffered formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5–6 μm in thickness were cut and stained with hematoxylin and eosin (H&E). These sections were examined by photo microscopy for necrosis, steatosis and fatty changes of hepatic cells.^[27]

Statistical analyses

Experimental results are presented as the mean ± standard deviation (SD) of three parallel measurements. All statistical analysis is performed using Minitab graphical computer software package.

RESULTS

Anticancer activity

The % of inhibition of cancer cell growth was found to be maximum and minimum at the concentrations of 200 and 12.5 μg/ml of samples respectively. CE of *C. ternata* showed maximum inhibition of cancer cell growth against MCF-7 with an inhibition of 60.60% followed by K-562 with 41.6% and finally HeLa with an inhibition of 31.28% as shown in Figure-1. The cytotoxic activity of PE showed maximum % of inhibition against MCF-7 with 54.1%, followed by K-562 with 30.3% and minimum with 21.32% inhibition against HeLa cells as shown in Figure-2.

Hepatoprotective activity and biochemical studies

The protective potential of CE and PE of *C. ternata* was investigated with an aim to identify whether this plant had potential hepatoprotective medicinal activity. Changes in the activities of serum enzymes (SGPT, SGOT and ALP), total bilirubin and total protein content in the serum of CCl₄-induced liver damage in rats are reported in Table. 1.

Antioxidant studies

The effect of CE and PE of *C. ternata* on lipid peroxidation (expressed in terms of MDA levels), SOD and CAT levels in rat liver tissue are shown in Table. 2. SOD activity in CCl₄ toxic group (3.433 ± 0.5773 U/mg protein) was determined to be lower than in the normal group (7.0666 ± 0.5507 U/mg protein). SOD activities in plant extract treated groups (100 mg/kg b.w) were observed to be higher when compared to CCl₄ toxic group. Silymarin,

with a dose of 25 mg/kg restored the SOD levels to the normal group compared to CCl₄ treated toxic group. CAT activity of liver homogenate in CCl₄ treated toxic group (143.9 ± 4.630 U/mg protein) was found to be low compared to LP treated normal group (251.46 ± 3.00 U/mg protein). Higher levels of CAT activities were noticed in CE and PE treated groups (228.8 ± 5.787 U/mg protein and 206.5 ± 2.749 U/mg protein) than in the CCl₄ toxic group respectively. The Silymarin treated group almost restored the enzyme activity to the LP treated normal group level at the dose of 25 mg/kg. The TBARS was found to be maximum in CCl₄ treated group (8.486 ± 0.374 MDA

equivalents) compared to LP treated normal (1.476 ± 0.096 MDA equivalents) group. A decrease in the levels of MDA equivalents was noticed in the CE and PE treatment groups with 3.353 ± 0.228 and 5.226 ± 0.143 MDA equivalents respectively compared to CCl₄ toxic group. Silymarin treated levels were not significantly different to the normal group.

Histopathological studies

Histopathological studies confirmed the hepatoprotective effect of the CE and PE of *C.ternata* was shown in the Figure 3–7. The results of histopathological studies

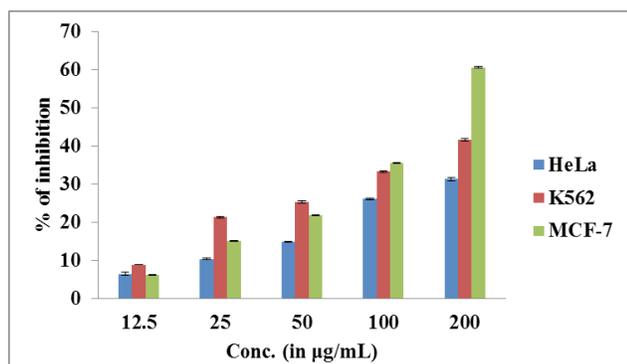


Figure 1. The effect of CE of *C.ternata* against different carcinogenic cell lines. Each is a value of mean ± standard deviation of triplicates.

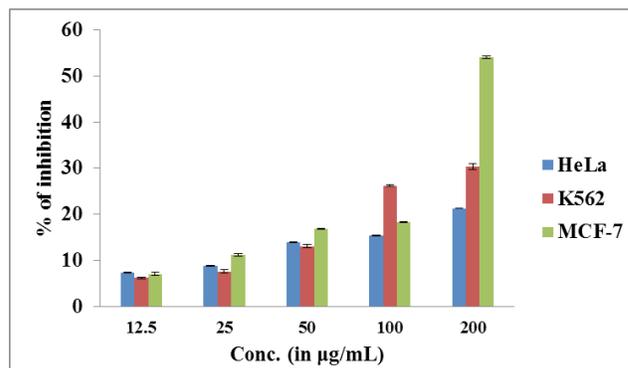


Figure 2. The effect of PE of *C.ternata* against different carcinogenic cell lines. Each is a value of mean ± standard deviation of triplicates.

Table 1: The effect of PE and CE of *C. ternata* on TB, SGPT, SGOT, ALP, total protein levels in rats toxicated with CCl₄.

Name of the group	TB	SGPT	SGOT	ALP	SERUM PROTEIN
G-I: LP treated normal control	0.533 ± 0.152	82.6 ± 10.59	76.3 ± 11.59	354 ± 30.26	5.243 ± 0.205
G-II: CCl ₄ treated toxic	2.6 ± 0.255*	123.33 ± 5.77*	121 ± 11.26	880.6 ± 27.22*	3.77 ± 0.335*
G-III: CCl ₄ + CE of <i>C.ternata</i>	1.16 ± 0.152**	102.33 ± 7.63**	98.33 ± 2.51**	475 ± 13.2**	4.59 ± 0.165**
G-IV: CCl ₄ + PE of <i>C.ternata</i>	1.533 ± 0.305	115.6 ± 2.516**	109.33 ± 2.5	553.3 ± 10.4**	4.89 ± 1.00**
G-V: CCl ₄ + Silymarin treated	0.566 ± 0.057**	78.66 ± 2.50**	71.33 ± 2.51**	255 ± 18.027**	5.81 ± 0.106**

Values are means ± standard deviation (n = 4). p values calculated by ANOVA and p < 0.05 values are considered statistically significant.

**p < 0.05 compared with CCl₄ treated toxic control group.

*p < 0.005 compared with LP treated normal group.

Table 2: Stress inhibitory (Antioxidant) activity of CE and PE of *C. ternata* on CCl₄ induced toxicity.

Name of the sample	SOD (U/mg of Protein)	CAT (U/mg of Protein)	TBARS MDA levels
G-I: LP treated normal control	7.066 ± 0.550	251.4 ± 3.00	1.476 ± 0.096
G-II: CCl ₄ treated toxic	3.433 ± 0.577*	143.9 ± 4.630*	8.486 ± 0.374*
G-III: CCl ₄ + CE of <i>C.ternata</i>	6.433 ± 0.65**	228.8 ± 5.787**	3.353 ± 0.228**
G-IV: CCl ₄ + PE of <i>C.ternata</i>	4.80 ± 0.3**	206.5 ± 2.749**	5.226 ± 0.143**
G-V: CCl ₄ + Silymarin treated	7.80 ± 0.360**	257.0 ± 2.542**	1.576 ± 0.140**

Values are means ± standard deviation (n = 4). p values calculated by ANOVA and p < 0.05 values are considered statistically significant.

**p < 0.05 compared with CCl₄ treated toxic control group.

*p < 0.005 compared with LP treated normal group.

clearly demonstrated that the normal liquid paraffin treated control showed the normal polyhedral hepatocytes with granular cytoplasm and each cell has a centrally located nucleus with one or two nucleoli in addition to a number of chromatin particles (Figure-3). CCl_4 induced hepatotoxic liver revealed the cloudy swelling, fatty degeneration with displacement of the nucleus of hepatocytes and necrosis of cells were also clearly seen (Figure-4). Treatment of the CCl_4 toxicated group with CE and PE of *C.ternata* exhibited a significant improvement of hepatocellular architecture and is evident of considerable reduction in necrosis (Figures-5 & 6). The CCl_4 induced toxicated group treated with Silymarin showed a good recovery with the absence of necrosis and fatty degeneration as shown in Figure-7.

DISCUSSION

Combating against cancer is a prime importance today. Many interdisciplinary researches are giving good efforts in scope to fight this disease but complete is still to come in world medicine. The medicinal approaches used to treat the disease have wide and severe side effects. Hence, an alternative medicine, i.e., the use of medicinal preparations to arrest the spread over of the disease^[28] is necessary. Generally, plant formulations capable of inhibiting the cell proliferations inducing apoptosis or modulating signal transduction are used in the treatment of cancer. Recently, it was reported that plant derived agents suppress the tumor progression and induces apoptosis in cancer cells and there by enhances the immunity and act as anticancer drugs.^[29] In the present study, we observed that both PE and CE of *C.ternata* showed significant levels of inhibition of cell proliferation and thereby involved in the reticence of stress related cancer. CE is more potent and significant than PE of *C.ternata*.

Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxin. During the metabolism, CCl_4 is bio transformed into trichloromethyl radical by the action of cytochrome p450 dependent enzyme in the liver, which reacts with molecular oxygen to form trichloromethyl peroxy radical.^[30] Both trichloromethyl and trichloromethyl peroxy radicals induce lipid peroxidation followed by pathological changes such as elevated levels of serum marker enzymes such as SGOT, SGPT, bilirubin and ALP.^[31] Necrosis or membrane damage caused due to lipid peroxidation releases the liver enzymes into circulation and therefore the levels can be measured in the serum. Our results clearly showed that the level of serum marker enzymes SGPT, SGOT, ALP and total bilirubin were

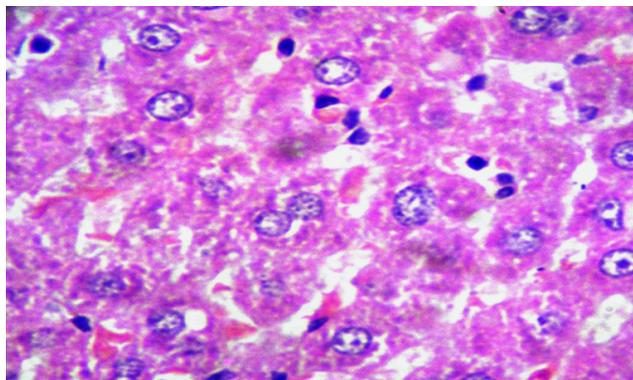


Figure 3. Showing group-I: LP treated normal group having normal hepatocytes with granular cytoplasm and each cell has a centrally located nucleus.

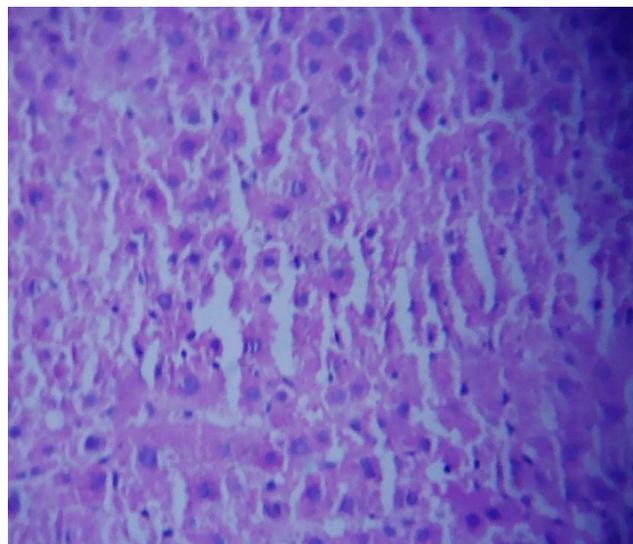


Figure 4. Showing group-II: CCl_4 treated toxic group revealed the cloudy swelling, fatty degeneration with displacement of the nucleus of hepatocytes and necrosis of cells was clearly seen.

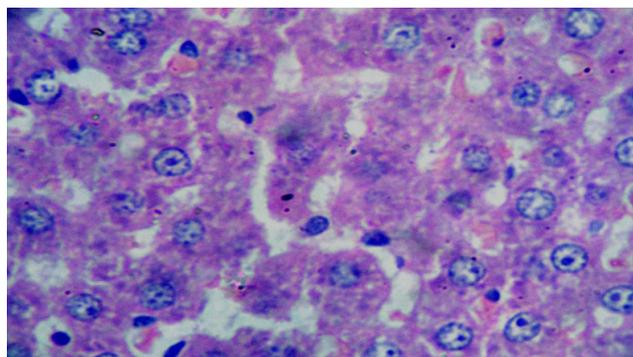


Figure 5. Showing group-III: CCl_4 intoxicated group animals treated with CE of *C.ternata*. This group showed almost normal hepatocytes with moderate portal system and normal lobular architecture.

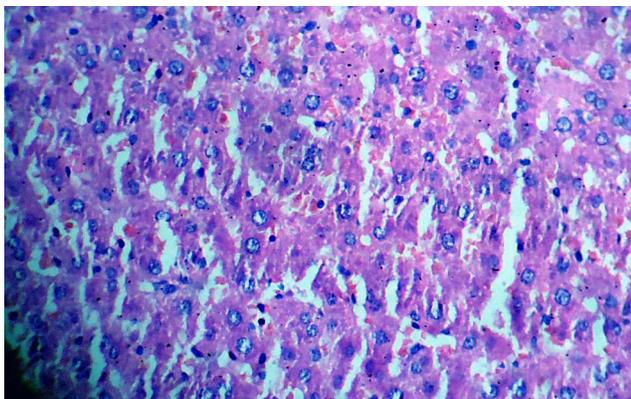


Figure 6. Showing Group-IV: CCl₄intoxicated group animals treated with PE of *C.ternata*. This group showed recovery from hepatic damage.

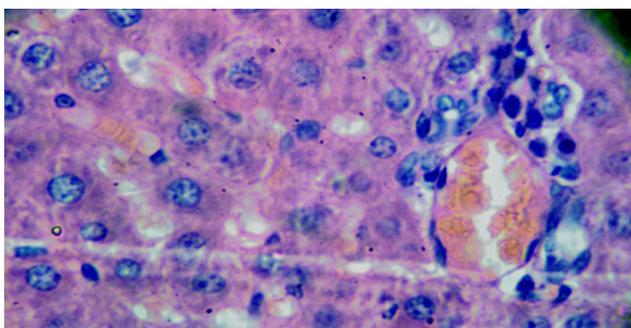


Figure 7. Showing Group-V: CCl₄intoxicated group animals treated with Silymarin. This group showed normal hepatic cells each with well-defined cytoplasm, nucleus, nucleolus and well brought out central vein.

significantly increased whilst the total protein content was significantly decreased in CCl₄-induced liver damage toxic group compared to LP treated normal control group. However, treatment with 100 mg/kg b.wof CE and PE of *C. ternata* induced a significant fall in the levels of serum transaminases (SGPT, SGOT), ALP and total bilirubin as well as raise in total protein content was noticed in comparison with toxic control group. These results are undoubted evidence that both CE and PE of *C.ternata* protected the structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells caused by CCl₄. Silymarin, a well-established hepatoprotective drug used as positive control. Treatment with Silymarin showed significant fall in the levels of serum hepatic marker enzymes and also increases the total protein content in comparison with toxic control group. Free-radical (ROS) mediated oxidative stress is responsible for many diseases including rheumatoid arthritis, cardiovascular disorders, cystic-fibrosis, inflammation, neurodegenerative diseases, AIDS, and cancer.^[32] Living cells have a number of mechanisms

to protect themselves from the toxic effects of ROS. Antioxidant defense mechanisms are maximally involved in the inhibition of ROS. SOD removes superoxide (O₂²⁻) by converting it to H₂O₂ and O₂, which can be rapidly converted to water by CAT and GPX.^[33] It is well documented that both SOD and CAT are the antioxidant enzymes play an important role in the elimination of ROS liberated during the peroxidative process of xenobiotics in liver tissues. The observed increase of SOD and CAT activities in CE and PE treatment groups compared to toxic group suggests that CE and PE of *C. ternata* have an efficient protective mechanism in response to ROS. These findings also indicate that both CE and PE may be associated with decreased oxidative stress and free radical mediated oxidative damage. The localization of radical formation resulting due to lipid peroxidation, measured as TBARS and expressed in the form of MDA equivalents. In our study, the MDA levels were observed to be high in the CCl₄ treated toxic group compared to LP treated normal group. The elevated MDA levels in toxic group may be due to enhanced lipid peroxidation leading to tissue damage and failure of the antioxidant mechanism. Treatment with CE and PE of *C. ternata* significantly reversed these changes. Histopathological studies also revealed that both PE and CE of *C.ternata* might play role in regulating hepatotoxicity. Hence, the mechanism of hepatoprotection of CE and PE of *C.ternata* may be due to its antioxidant effect. The cytotoxic, antioxidant and hepatoprotective effects were due to the presence of various phytochemical compounds including tannins, flavonoids, polyphenols and alkaloids as reported from previous studies.^[16,17] A number of scientific reports indicated the phytochemical compounds and their derivatives may be responsible for the antitoxic properties including cytotoxic, antioxidant and hepatoprotective activities.

CONCLUSION

Both CE and PE of *C.ternata* have an ability to protect the liver from CCl₄ induced liver damage. In addition, they also exhibited anticancer activity. Both extracts may be associated with decreased oxidative stress and free radical mediated tissue damage because of antioxidant and radical scavenging properties. Further, investigation is underway to determine the active principle involved in the process of hepatoprotection and antioxidant activity.

COMPETING INTERESTS

The authors declare that they have no competing interests

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REFERENCES

1. Harman D. Free-radical theory of aging, increasing the functional life span. *Ann Ny Acad Sci.* 1971; 239:1–15.
2. Dizdaroglu M, Jaruga P. Mechanisms of free radical-induced damage to DNA. *Free Radical Res.* 2012; 46(4):382–419.
3. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol.* 1990; 186:1–85.
4. Blot WJ, Lyji Taylor PR. Nutrition intervention trials in Linzian. China: Supplementation with cancer incidence, and disease specific mortality in general population. *Nat Can Inst.* 1993; 85:1483–91.
5. Rosa MP, Gutierrez, Rosario VS. Hepatoprotective and Inhibition of Oxidative stress in liver of *Prostechea michuacana*. *Rec Nat Prod.* 2009; 3:146–51.
6. Ravishankar SB, Bhavsar GC. Plants with hepatoprotective activity. *Ind Drugs.* 1993; 30:363–5.
7. Maheswari C, Maryammal R, Venkatanarayanan R. Hepatoprotective activity of *Orthosiphon stamineus* on liver damage caused by paracetamol in rats. *Jor J of Bio Sci.* 2008; 1:105–8.
8. Mitra SK, Seshadri SJ, Venkataranganna MV, Gopumadhavan S, Udupa VU, Sarma DN. Effect of HD-03-a herbal formulation in galactosamine-induced hepatopathy in rats. *Ind J Physio Pharmacol.* 2000; 44:82–6.
9. Mclanghlin JL. Bench top bioassay for the discovery of bioactive compounds in higher plants. *Brenena.* 1990; 29:50–4.
10. Evans WC (eds); Trease and Evans Pharmacognosy. WB.Saunders, Edinburgh. 2002; 475.
11. Jain NN, Ohal CC, Shroff RH, Somani RS, Kasture VS, Kasture SB. *Clitoria ternata* and the CNS. *Pharmacol Biochem Behav.* 2003; 75: 529–36.
12. Devi BP, Boominathan R, Mandal SC. Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternate* root. *Fitoterapia.* 2003; 74:345–9.
13. Kazuma K, Noda N, Suzuki M. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochem.* 2003; 62:229–37.
14. Terhara N, Toki K, Saito N, Honda T, Mastui T, Saojima Y. Eight new anthocyanins, ternatins C1–C5 and D3 and preternatins A3 and C4 from young *clitoria ternata* flowers. *J. Nat. Prod.* 1998; 61:1361–67.
15. Nithianantham K, Murugesan S, Yeng Chen, Lachimanan YL, Jothy SL, Sasidharan S. Hepatoprotective potential of *Clitoria ternata* leaf extract against paracetamol induced damage in mice. *Molecules.* 2011; 16:10134–45.
16. Bhaskara Rao D, Koteswara Rao P, Sumitra DJ, Raghava Rao T. Phytochemical screening and antioxidant evaluation of some Indian medicinal plants. *J. Pharm. Res.* 2011; 4:2082–4.
17. Bhaskara Rao D, Ravi Kiran Ch, Madhavi Y, Koteswara Rao P and Raghava Rao T. Evaluation of antioxidant potential of *C.ternata L.* and *E.prostrata L.* *Ind. J. Biochem & Biophy.* 2009; 26:247–52.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol Methods.* 1983; 65:55.
19. Manoj B, Aqueed K. Protective effect of *Lawsonia alba* Lam., against CCl₄-induced hepatic damage in albino rats. *Ind J Expt Biol.* 2003; 41: 85–7.
20. Malloy HT, Evelyn KA. The determination of bilirubin with the photometric colorimeter. *J Biol Chem.* 1937; 119:481–90.
21. Bergmeyer HU, Scheibe P and Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clin. Chem.* 1978; 24:58–61.
22. King J. The hydrolases-acid and alkaline phosphatase, *Practical Clinical Enzymology.* Van, D (ed.) Nostrand company Ltd, London. 1965; 191–208.
23. Beauchamp C and Fridovich I. Superoxide dismutase: Improved assay and an assay applicable to acrylamide gels. *Anal Biochem.* 1971; 44(1): 276–87.
24. Radhakrishnan TM, Sarma PS. Intracellular localization and biosynthesis of catalase in liver tissues. *Curr Sci.* 1963; 32:1749.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin- phenol reagent. *J Biol Chem.* 1951; 193:265–75.
26. Ohkawa H, Onishi, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Anal Bio Chem.* 1979; 95:351–8.
27. Gallighar AE, Kozloff EN. *Essential of practical micro-technique*, 2nd Edn. Lea and Febiger, Philadelphia. 1971; 77:210.
28. Senthilkumar Natesan, Shrishailappa Badami, Santosh kumar H. Dongre, Ashok Godavarthi. Antitumor activity and antioxidant status of the methanol extract of *Careya arborea* bark against Dalton's lymphoma Ascites-induced Ascitic and solid tumor in mice. *J of Pharm Sci.* 2007; 103:12–23.
29. Devita VT. The relationship between tumor mass and resistance to chemotherapy: Implication for surgical adjuvant treatment of cancer. *Cancer.* 1983; 51:1209–20.
30. Raucy JL, Kraner JC, Lasker J. Bioactivation of halogenated hydrocarbons by cytochrome P 450 E1. *Crit Rev Toxicol.* 1993; 23:1–20.
31. Zimmerman HJ, Seeff LB. *Enzymes in hepatic disease*, Lea and Febiger. Philadelphia. 1970:24–26.
32. Halliwell H. Free radicals, antioxidants and human disease: Curiosity, cause or consequence? *Lancet.* 1994; 344:721–4.
33. Halliwell B, Gutteridge JM, Cross CE. Free radicals, antioxidants, and human disease: where are we now? *J Lab Clin Med.* 1992; 119:598–620.