Advances in Bioresearch
Adv. Biores., Vol 15 (5) September 2024: 312-322
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.5.312322

Advances in Bioresearch

ORIGINAL ARTICLE

Evaluation of Hepatoprotective Activity of *Macrothelypteris ornata* Against CCl_4 – Induced Hepatoxicity in Rats

Aswini G, Lavanya Yaidikar*
Seven Hills College of Pharmacy, Tirupati – 517561, Andhra Pradesh, India
***Correspondence Author**: Lavanya Yaidikar,
Email: ylavanya.balaji@gmail.com

ABSTRACT
Liver is one of the important organ for metabolic functions and detoxification of various endogenous and exogenous substances. And its damage is associated with the substances like paracetamol, thioacetamide, alcohol, phenylhydrazine, sodium valproate etc., which remains challenging. It is important to protect liver from such encountered damage from drugs, thereby can improve therapeutic benefit. This can be achieved by use of medicinal plants with potential source of antioxidant activity. Macrothelypteris ornata, a fern with rich antioxidant activity and we evaluated the potential of ethanolic extract (EEMO) protection against carbon tetrachloride induced hepatotoxicity in rats which were measured by estimating biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin and total protein. Liver markers of Lipid peroxidation (LPO), reduced glutathione (GSH) and catalase (CAT) were also measured which was significantly reversed with the treatment of EEMO. The results have clearly indicated that EEMO exhibited hepatoprotection which was evident from serum and tissue biochemical evidences, as supported by histological evidences.
***Keywords**: Hepatoprotection, Carbon tetrachloride, Macrothelypteris ornata.*

Received 24.05.2024 Revised 01.07.2024 Accepted 11.09.2024

How to cite this article:
Aswini G, Lavanya Yaidikar. Evaluation Of Hepatoprotective Activity of *Macrothelypteris ornata* Against Ccl_4 – Induced Hepatoxicity in Rats. Adv. Biores., Vol 15 (5) September 2024: 312-322.

INTRODUCTION

The most important organ concerned with the biochemical activities in the human body is liver (1,2). It detoxicates different toxic substances by using a phenomenon called biotransformation. In the maintenance of metabolic functions and detoxification from various exogenous and endogenous challenges, like xenobiotics, drugs, viral infections, bacterial infections and chronic & acute alcoholism liver act as a paramount organ (3,4). During this phenomenon, liver subjected to wear and tear mechanism with various chemicals and drugs like Paracetamol, Carbon tetrachloride, Thioacetamide, alcohol and finally it leads to different diseases like hepatitis, cirrhosis, alcohol related disorders and liver cancer (5,6). Once liver damage was occurred, it is associated with cellular apoptosis, increase tissue lipid peroxidation and depletion in the tissue GSH levels along with this elevation in serum levels of various biochemical parameters like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase (7-9). There is tremendous research is going on now a day for management of liver disorders in a precise manner on herbal medicines which are claimed to possess hepatopreotective activity are extremely a prosperous source all over the world as and 80% of the world population majorly in the developing countries for primary health care they are using herbal medicine only. Due to eco-friendly nature of herbal drug products from ancient times for some age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, they used herbal medicine only. From hundreds of years, before the development modern medicine traditional medicine (including herbal drugs) as therapeutic practices that have been in existence and are still in use today also (10-13). *Macrothelypteris ornata* is a species of fern which is native to tropical and subtropical Africa and Asia belonging to the family Thelypteridaceae. It has been introduced into other areas, including large parts of North and South America. It contains flavonoids which have been investigated for possible medicinal

value. In traditional medicine, M. ornate leaves and roots have a wide range of reputed medicinal application (14). The aerial parts are used for granulation, healing, and reducing odor in chronic skin ulcers by traditional healers in Pakistan, India, and China. M. ornate is traditionally, used in Chinese folk medicine for the treatment of edema for patients suffering from kidney/bladder problem due to its satisfactory therapeutic effectiveness. Hence, the present study was undertaken to evaluate the hepatoprotective activity of ethanolic extract of *Macrothelypteris ornata* against carbon tetrachloride induced hepatotoxicity in rats.

MATERIAL AND METHODS

Plant Material and Extraction:

The aerial parts of *Macrothelypteris ornata* were collected from different sources and authenticated by Dr. K. Madhava Chetty, Professor, Dept. of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our research laboratory for further reference. The collected materials were washed with water and shade dried for one week and then the dried aerial parts were pulverized by using a mechanical grinder to obtain coarse powder. The powdered plant material (500 g) was extracted with ethanol (90% v/v) for 48hrs using Soxhlet extractor. The extract obtained was evaporated under vacuum to remove the solvent completely and concentrated to obtain a dark greenish residue (11.56 g) (15,16).

Preliminary Phytochemical Studies

A plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of a compounds like glycosides, alkaloids, volatile oils, saponins, etc., that exert a physiological effect. The compounds that are responsible for therapeutic effects are usually the secondary metabolites. A systematic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism.

The ethanol extract of *Macrothelypteris ornata* aerial parts subjected to preliminary phytochemical screening for the detection of various phytoconstituents (17-19).

1. Tests for alkaloids

i) Amyl alcohol test:

About 300 mg of extract is digested with 2 M HCl. Acidic filtrate is mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink colour which indicates the presence of alkaloids.

ii) Using reagents:

a) Mayer's reagent

Produces white to buff coloured precipitate with alkaloids.

b) Wagner's reagent

Produces reddish brown precipitate with alkaloids.

c) Dragendorff's reagent

Gives orange brown precipitate with alkaloids.

d) Hager's reagent

Gives characteristic yellow crystalline precipitate with many alkaloids.

2. Test for carbohydrates

a) Molisch's test

To aqueous or alcoholic solution of the substance in a test tube add 10% alcoholic solution of α-napthol. Shake well and add a few drops of concentrated sulphuric acid along the side of the test tube. A violet ring at the junction of two liquids confirms the presence of carbohydrates.

b) Fehling's test

Add 2 ml of Fehling's solution A and 2 ml of Fehling's solution B to 2 ml of liquid extract in a test tube and boil. Formation of yellow or brick red precipitate confirms presence of reducing sugars.c) Benedict's test

Add 5 ml of Benedict's reagent to 3 ml of test solution in a test tube and boil on a water bath. Appearance of brick red precipitate at the bottom of the test tube shows presence of monosaccharides.

3. Test for glycosides

A) TEST FOR CARDIAC GLYCOSIDES (KELLER KILLIANI TEST)

To an extract of the drug in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added. A reddish brown colour is formed at the junction of two layers and the upper layer turns bluish green. The test confirms presence of cardiac glycosides with presence of digitoxose as the glycone moiety.

b) Test for anthraquinone glycosides

i) Borntrager's test
Boil 0.1 gm of the powdered drug with 5 ml of 10% sulphuric acid for 2 min Filter while hot, cool the filtrate and shake gently with equal volume of benzene. Pipette out and transfer the benzene layer to a clean test tube. Add about half of its volume of ammonia solution and shake. The ammoniacal layer acquires pink to red colour due to presence of free anthraquinones.
ii) Modified Borntrager's test
The C-glycosides of anthraquinones require more drastic conditions for hydrolysis and thus a modification of the above test is used. Ferric chloride and hydrochloric acid are used to affect oxidative hydrolysis. 0.1 g of the drug is boiled with 5 ml of dilute hydrochloric acid and 5 ml of 5% solution of ferric chloride for five minutes, cooled, and filtered. This filtrate is shaken with benzene. The benzene layer is separated and an equal volume of dilute solution of ammonia is added. The ammoniacal layer shows pink to red colour.

4. Test for saponins

A) Foam test
Boil 300 mg of extract with 5 ml water for two minutes. Mixture is cooled and mixed vigorously and left it for three minutes. The formation frothing indicates the presence of saponins.
b) Haemolysis test
To 2 ml of 1.8% sodium chloride solution in two test tubes, 2 ml distilled water is added to one and 2 ml of 1% extract to the other. 5 drops of blood is added to each tube and gently mixed with the contents. Haemolysis is observed under a microscope.
c) Steroidal saponin:
Extract hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.
d) Triterpenoidal saponin:
Extract hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for triterpenoids.

5. Test for gums and mucilages

a) Precipitation with 95% alcohol:
Gums and mucilage precipitate with addition of 95% alcohol, being insoluble in alcohol.
b) Molisch's test:
To aqueous or alcoholic solution of the substance in a test tube add 10% alcoholic solution of α-Napthol. Shake well and add a few drops of concentrated sulphuric acid along the side of the test tube. A violet ring at the junction of two liquids confirms the presence of carbohydrates, gums and mucilages.
Ruthenium red test:
Dissolve 0.8 g of ruthenium red in 10 ml of 10% solution of lead acetate. It stains mucilage to red colour.

6. Test for tannins and phenolic compounds

a) With Ferric chloride
A 1% w/w solution of ferric chloride gives blue/green/brown colour.
b) With Lead acetate
Tannins are precipitated with lead acetate solution.
c) With Gelatin solution
To a solution of extract, aqueous solution of gelatin (1%) and sodium chloride (10%) are added. A white to buff coloured precipitate is formed if tannins are present.

7. Test for steroids and sterols

a) Liberman Burchard reagent
To about 2 ml of a solution of extract in chloroform in a dry test tube, add few drops of acetic anhydride and mix well. 1 ml of Conc. H_2SO_4 is added from side of the test tube. A reddish-brown ring is formed at the junction of two layers.
b) Salkowski's test
To 5 ml of a solution of extract in chloroform in a dry test tube add gently along the sides, an equal volume of concentrated sulphuric acid. Observe the upper chloroform layer and the lower acid layer. The acid layer develops a yellow colour with a green fluorescence. The chloroform layer will give a play of colours first from bluish red to gradually violet red.
8. Test for proteins and amino acids
a) Biuret test
To 2 ml of extract, 2 ml of 10% sodium hydroxide solution and 2 to 3 drops of 1% copper sulphate solution is added and mixed. Appearance of violet or purple colour confirms presence of proteins.
b) Ninhydrin test

To 2 ml of extract add 0.5 ml of ninhydrin solution. Boil for 2 min and cool. Appearance of blue colour confirms presence of proteins.

c) Xanthoproteic test

To 2 ml of extract add 1 ml of concentrated nitric acid, boil, cool and add 40% sodium hydroxide solution drop by drop. Appearance of coloured solution indicates presence of proteins.

c) Millon's test

To 2 ml of extract add 2 ml of Millon's reagent, boil, cool and add few drops of sodium nitrite solution. Appearance of red precipitate or colour indicates presence of proteins.

9. Test for Triterpenoids

a) Salkowski test

Lower ring turns golden yellow colour.

b) Liberman Burchard test

Red ring is produced at the junction.

*d)*Ischugajiu test

Excess acetyl chloride and a pinch of zinc chloride are added to the chloroform solution, kept aside for the reaction to subside and warmed on a water bath. Cosin red colour is produced.

d) Brickorn and Brinar test

To the chloroform solution of the extract, few drops of chlorosulfonic acid in glacial acetic acid (7:3) are added. Red colour is produced.

e) Tin and thionyl chloride test

To the extract dissolved in chloroform, add piece of metallic tin. Add 1 drop of thionyl chloride. If pink colour develops then triterpenoids are present.

f) Sulphuric acid test

About 300 mg of extract is mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution is then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicates the presence of triterpenes.

10. Test for Flavonoids

a) The extract dissolved in water, filtrate treated with sodium hydroxide, a yellow colour is observed if flavonoids are present. A drop of concentrated sulphuric acid when added to the above, the yellow colour disappears.

b) Shinoda test

To the aqueous or alcoholic solution of the extract, add a piece of magnesium ribbon and few drops of concentrated hydrochloric acid. A pink/magenta colour develops which indicates presence of flavonoids.

c) Ferric chloride test

Few drops of neutral ferric chloride solution are added to little quantity of alcoholic extract. A blackish green colour is produced indicates phenolic nucleus.

d) Lead acetate test

Few drops of 10% lead acetate solution are added to alcoholic solution of the extract. Yellow precipitate is observed.

e) Zinc-hydrochloric acid reduction test

The alcoholic solution treated with a pinch of zinc dust and few drops of concentrated hydrochloric acid. Magenta colour is produced after few min.

Acute toxicity study

The acute toxicity studies were conducted as per the OECD guidelines 423, where the limit test dose of 2000 mg/kg, p.o., used. Immediately after dosing, the animals were closely observed for the initial 4 h after the administration and then once daily during the following days. The behavioural changes closely observed for were hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep and coma. They were then kept under observation up to 14 days after drug administration to find out the mortality if any. One-tenth and one-fifth of the maximum tolerated dose (200 and 400 mg/kg, body weight, p.o.) of the ethanol extract of M. ornata aerial parts were selected for analgesic, antipyretics and anti-inflammatory activities studies (20,21).

Assessment of Hepatoprotective Activity

Experimental groups

Hepatoprotective effect was evaluated against CCl_4 induced hepatotoxicity (22,23). Animals were divided into five groups (n=6). Group I (Normal Control) animals received single dose of water (25 mL/kg, p.o.) daily for 7 days and received olive oil (8 mL/kg, i.p.) on 7^{th} day. Group II (Toxicant control) animals received 0.2% CCl_4 in olive oil (8 mL/kg, i.p.) on 7^{th} day intraperitoneally and were also administered with single dose water (25 mL/ kg, p.o.) once daily for 7 days. Group III animals were treated with

standard drug silymarin (100 mg/kg, p.o.) once daily for 7 days whereas groups IV and V animals were treated with EEMO at doses of 300 and 600 mg/kg, p.o., dissolved in 2% gum acacia once daily for 7 days respectively. Similarly, groups III to V animals simultaneously treated with 0.2% CCl_4 in olive oil (8 mL/kg, i.p.) on 7th day after 1 h of administration of the silymarin and EEMO. After 24 h of treatment, blood from all animals were collected by retro-orbital puncture and after that the animals were sacrificed. Blood was allowed to clot and centrifugation was performed at 3500 rpm for 15 min at 4°C to separate the serum which was used for the assay of biochemical marker enzyme. Liver tissue samples were taken from the left liver lobe and they were cut into two pieces. One piece was fixed in 10% formalin solution for 24 h for pathological examination; the other piece was used in assessment of lipid peroxidation assay.

Biochemical estimations

Serum which was separated by centrifugation were used to determine the biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT),alkaline phosphatase (ALP), total bilirubin and total protein using commercially available kits. Liver weight was also determined by recording the weight of liver with respect to body weight per 100 gram (24,25).

Assay for hepatic antioxidant activities

Lipid peroxidation

Liver tissue (900 mg) was taken from each experimental animal(s) and then it was washed in normal saline and soaked in filter paper. Homogenization of the tissues were done using 3.0 mL (0.15 M) tris HCl buffer (pH 7.4) and then centrifuged for 1 h at 3000 rpm at 4°C.The resultant supernatant was collected and estimated for lipid peroxidation. LPO is determined by measuring the amounts of malondialdehyde (MDA) produced primarily which was expressed as µ mole/g liver tissue (26).

Glutathione content

Liver homogenate (30% w/v) was prepared in 0.15 M Tris- HCl buffer (pH 7.4) and proteins were precipitated by adding trichloroacetic acid. The following samples were centrifuged for 1 h at 15000 rpm at 4°C. The supernatant obtained was used for determination of glutathione content and it was expressed as µg/g of liver tissue (27).

Catalase activity

Homogenization of 900 mg of liver tissue was done in 3.0 mL M/150 phosphate buffer in ice and then centrifuged for 1 h at 30000 rpm at 4°C. The supernatant was collected and catalase activity was measured (28).

Histological observation

Fixatives such as picric acid, formaldehyde and 40% glacial acetic acid were used to fix the washed liver for 24 h and they were then dehydrated with alcohol. The liver tissue was cleaned and was embedded in paraffin (melting point 58-60°C). 3-5 µm sections of the liver tissue were cut and stained with haematoxylin and eosin E. After staining they were observed under photomicroscope and the following morphological changes like hepatic cell necrosis, ballooning degeneration, fatty changes or inflammatory cell infiltration and congestion were observed.

Statistical Analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's t-test. A p-value <0.05 was considered to be significant. All the values were expressed as Mean ± SEM.

RESULTS

Preliminary phytochemical tests

Preliminary phytochemical screening of the ethanol extract from *Macrothelypteris ornata* aerial parts contains sterols, flavonoids, saponins, proteins, reducing sugar, tannins and phenolic compounds (Table 1).

Acute oral toxicity studies

No mortality or morbidity was observed in animals through the 14 day period following single oral administration. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsions, salivation, diarrhoea, lethargy or unusual behaviours such as self-mutilation, walking backward etc. were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was all normal. There was no significant difference in body weights between control and treatment groups. Food and water intake showed daily fluctuations within the range of control animals. This indicates that the ethanol extract from M. ornata aerial parts was safe upto a single dose of 3000 mg/kg, p.o., body weight in experimental mice.

Hepatoprotective evaluation

Effects of EEMO on serum marker enzymes (SGPT, SGOT and ALP), total protein, total bilirubin and liver weight

As indicated from the results (Table 2 and Table 3), CCl_4 intoxicated animals showed an increase in the activities of SGPT, SGOT, ALP and total bilirubin when compared to the normal control group. Pre-treatment of animals with EEMO at the doses of 300 and 600 mg/kg, p.o., or silymarin 100 mg/kg, p.o., significantly decreased the level of serum marker enzymes (SGPT, SGOT and ALP) and total bilirubin compared to the CCl_4 intoxicated group. Whereas total protein level was decreased in the CCl_4 intoxicated group which was significantly restored to normal level when the animals were treated with EEMO at the doses of 300 and 600 mg/kg, p.o., or silymarin 100 mg/kg, p.o., (Table 2). Liver weight was enlarged in CCl_4 intoxicated group compared to normal group but it significantly regained its normal size when the animals were treated with EEMO at the doses of 300 and 600 mg/kg, p.o., or silymarin 100 mg/kg, p.o., (Table 3).

Effects of EEMO on antioxidant enzymes (LPO, GSH and CAT)

The result shows that there is an increase in levels of LPO which was measured in terms of malondialdehyde (MDA) levels in CCl_4 intoxicated group. Pre-treatment of the animals with EEMO at doses of 300 and 600 mg/kg, p.o., and silymarin (100 mg/kg, p.o.) significantly ($P<0.01$) decreased the levels of LPO compared to CCl_4 intoxicated group (Figure 1). The levels of GSH and CAT decreased in CCl_4 intoxicated group when compared to control but after pre-treatment of animals with EEMO (300 and 600 mg/kg, p.o.) or silymarin (100 mg/kg, p.o.) there was a significant rise in activity of GSH and CAT compared to CCl_4 intoxicated group (Figure 2 and 3).

Histopathological Observations

In normal control rats, no abnormal appearance or histopathological changes were observed in the liver (Figure 4A). When compared to the normal tissues, CCl_4 administration caused hepatic damage in rat liver, as demonstrated by hepatic cell necrosis, ballooning degeneration, fatty changes or inflammatory cell infiltration (Figure 4B). Treatment with silymarin almost restored the normal architecture of liver (Figure 4C) whereas treatment with EEMO decreased the abnormality of liver architecture caused by CCl_4 and restored the altered histopathological changes in a dose dependent manner (Figure 4D and Figure 4E).

Table 1: Preliminary phytochemical screening of ethanol extract from *Macrothelypteris ornata* aerial parts (EEMO).

Phytoconstituents	EEMO
Alkaloids	-
Carbohydrates	+
Gums and mucilages	+
Proteins and amino acids	+
Tannins and phenolic compounds	+
Phlobatannins	+
Steroids and sterols	+
Triterpenoids	-
Saponins	+
Flavonoids	+

(+) Present; (-) Absent

Table 2. Effect of EEMO on liver biomarkers in serum *(SGPT, SGOT, ALP and Total bilirubin levels).*

Groups	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Total Protein (g/L)	Total Bilirubin (mg/dL)
Normal Control	11.79±1.24	22.8±0.89	68.1±2.11	65.7±1.32	1.4±0.98
CCl_4 Control	94.6±2.07***	83.5±2.45***	375.9±4.78***	142.8±5.36***	6.5±1.5***
CCl_4+ Silymarin 100 mg/kg	41.8±1.88+++	35.4±1.66+++	114.5±2.65+++	62.7±2.85+++	1.02±0.54+++
CCl_4+ EEMO 300 mg/kg	62.4±2.56++	52.7±1.85++	246.5±3.78++	80.11±1.97+++	1.65±0.23+++
CCl_4+ EEMO 600 mg/kg	37.9±1.47+++	38.9±2.75+++	105.6±2.46+++	70.14±1.43+++	1.07±0.55+++

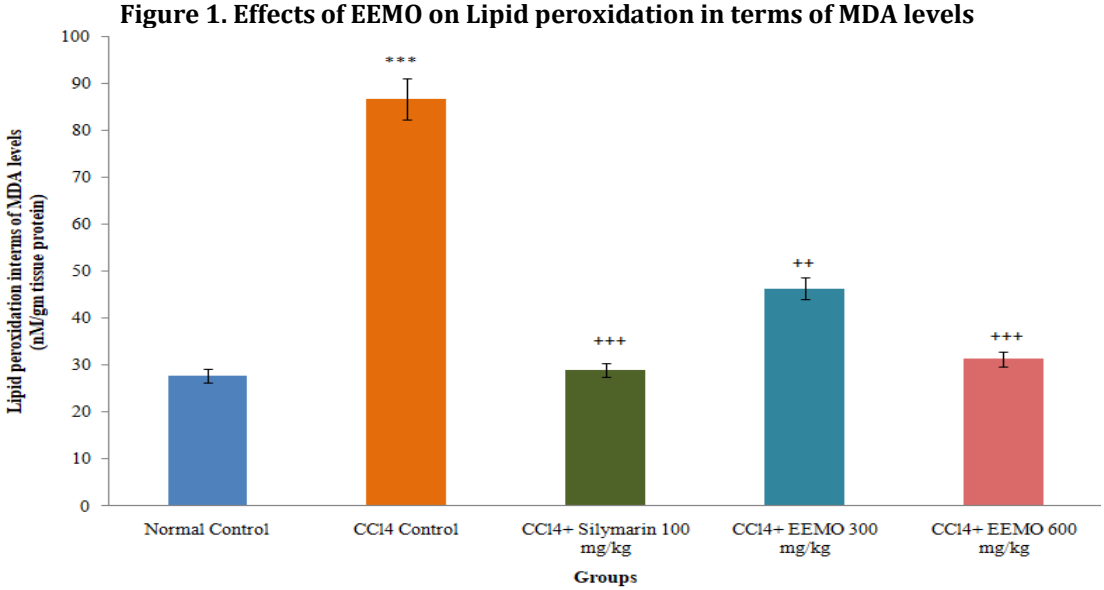
*Values are expressed as mean ± SEM (n = 6).; Data were analyzed by one way ANOVA followed by Dunnett's test.; ***p<0.001 as compared with normal control group; ++p<0.01, +++p<0.001 as compared with CCl_4 control group.*

Table 3: Effect of EEMO on liver weight

Groups	Liver weight (gms)
Normal Control	3.12±0.45
CCl_4 Control	6.89±1.27***
CCl_4+ Silymarin 100 mg/kg	2.3±0.12+++
CCl_4+ EEMO 300 mg/kg	3.8±1.55++
CCl_4+ EEMO 600 mg/kg	2.9±0.68+++

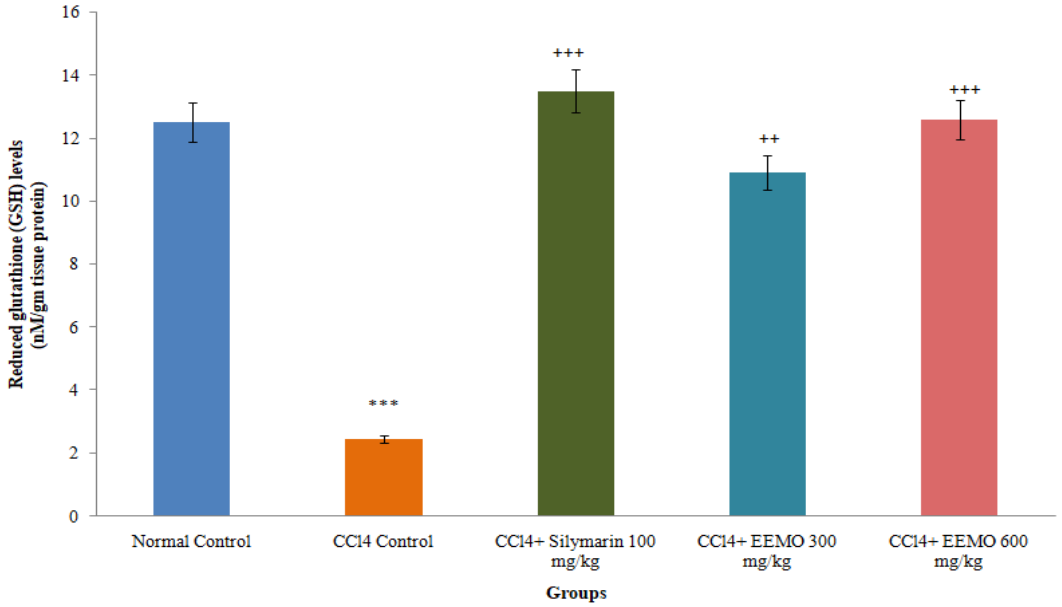
*Values are expressed as mean ± SEM (n = 6). Data were analyzed by one way ANOVA followed by Dunnett's test., ***p<0.001 as compared with normal control group; ++p<0.01, +++p<0.001 as compared with CCl_4 control group*

Figure 1. Effects of EEMO on Lipid peroxidation in terms of MDA levels



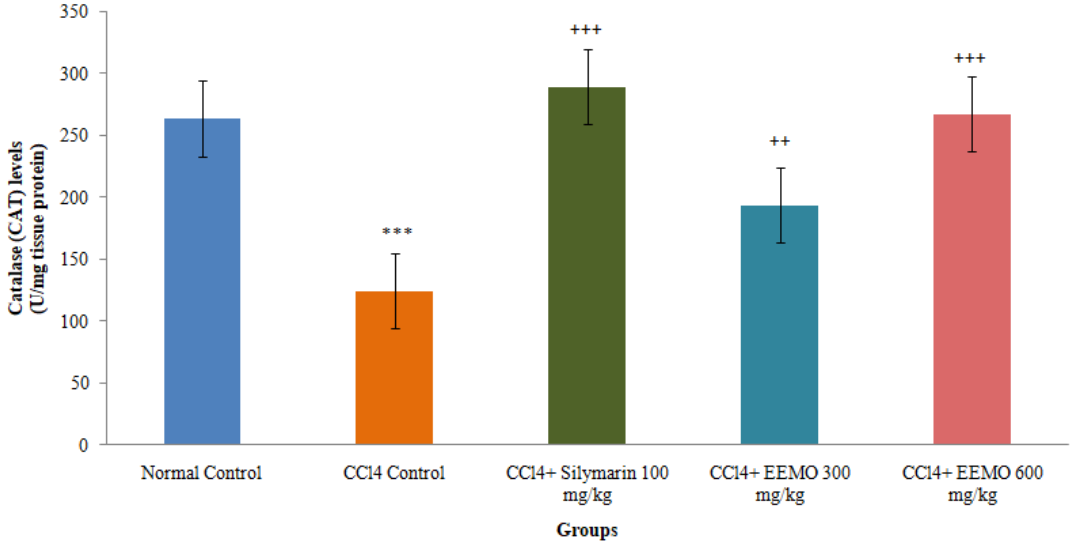
*Values are expressed as mean ± SEM (n = 6).; Data were analyzed by one way ANOVA followed by Dunnett's test.; ***p<0.001 as compared with normal control group;; ++p<0.01, +++p<0.001 as compared with CCl_4 control group*

Figure 2. Effects of EEMO on Reduced glutathione (GSH) levels

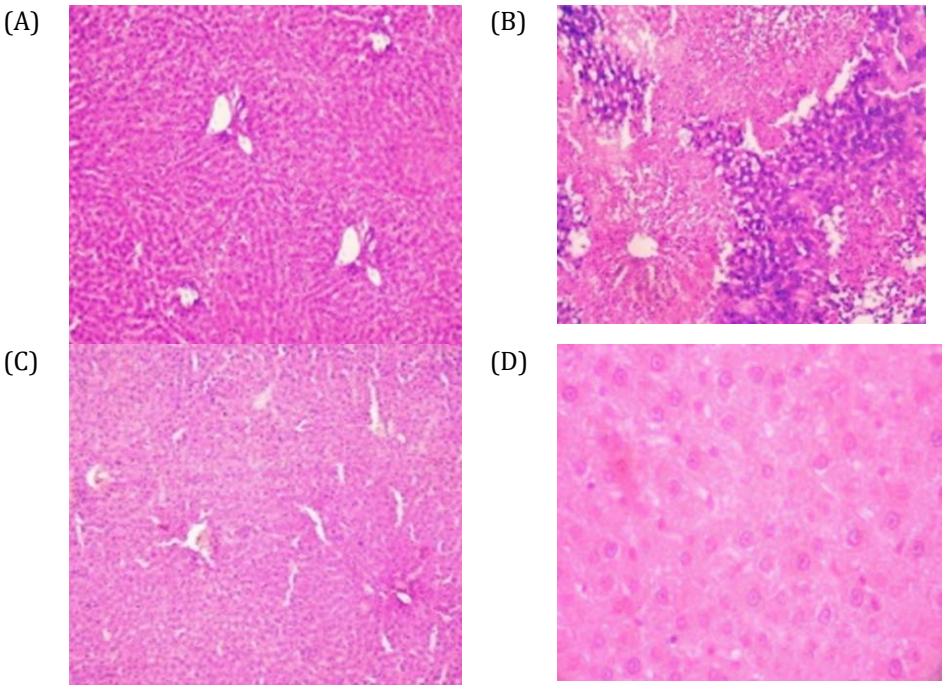


Values are expressed as mean ± SEM (n = 6).
*Data were analyzed by one way ANOVA followed by Dunnett's test. ***p<0.001 as compared with normal control group; ++p<0.01, +++p<0.001 as compared with CCl_4 control group.*

Figure 3. Effects of EEMO on Catalase (CAT) levels



*Values are expressed as mean ± SEM (n = 6). Data were analyzed by one way ANOVA followed by Dunnett's test. ***p<0.001 as compared with normal control group; ++p<0.01, +++p<0.001 as compared with CCl_4 control group.*



(E)

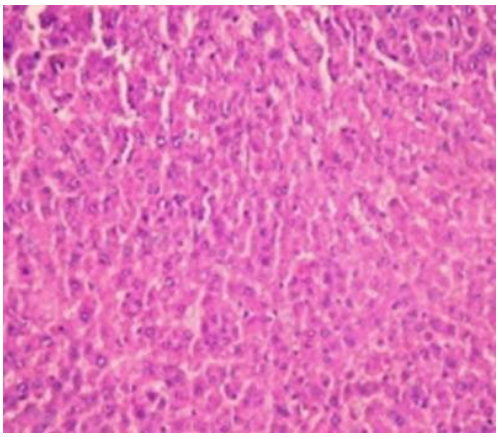


Figure 4. *Effect of EEMO on liver histological changes measured by haemoxylin and eosin staining* **(A)** *Vehicle control showing normal architecture of normal liver architecture.* **(B)** *hepatotoxic liver after treatment withCCl_4 showing hepatic cell necrosis, degeneration, inflammatory cell infiltration*. **(C)** *Liver treated with CCl_4 + silymarin (100 mg/kg) preserves almost the normal structure of the hepatocytes.* **(D)** *Liver treated with CCl_4 and EEMO (300 mg/kg) showing mild hepatic cell necrosis and infiltration of inflammatory cells.* **(E)** *Liver section treated with CCl_4 and EEMO (600 mg/kg) showing liver restoring to normalcy with little hepatic damage.*

DISCUSSION

Carbon tetrachloride is a hepatotoxin which is used extensively for inducing liver injury in various experimental models to elucidate the mechanisms underlying hepatotoxicity (29). CCl_4 mediated hepatotoxicity is developed from the biotransformation of CCl_4 by cytochrome P450 2E1 to the trichloromethyl free radical ($^{\bullet}CCl_3$) (30,31). Conversion of this free radical to a highly reactive species ($CCl_3O_2^{\bullet}$) is done by reaction with oxygen. Trichloromethyl peroxy radical binds covalently to cellular macromolecules and leads to a chain reaction of polyunsaturated fatty acids in the cytoplasmic membrane phospholipids, causing functional and morphological changes to the cell membrane, and finally, cell necrosis (32,33). An important mechanism involved in the protection against CCl_4-induced liver damage is the inhibition of excessive ROS production. MDA is widely used as a marker of lipid peroxidation and a major parameter for the status of oxidative stress (34). The hepatic MDA level increases under the enhancement of oxidative stress in a rodent model. Here in our study rats treated with CCl_4 exhibited a significant rise in MDA level (Lipid peroxidation assay) compared to control group. Treatment with EEMO significantly reduced the CCl_4 induced hepatic MDA elevation (35). Thus EEMO provides protective effect against CCl_4 induced liver damage in terms of preventing lipid peroxide formation and blocking oxidative chain reaction. The defense system in the body also includes small molecules such as GSH and vitamin E as well as antioxidant enzymes. The activities of hepatic antioxidants including GSH and CAT were declined by CCl_4 administration to rats, which is stated in earlier studies. Here our study revealed that treatment with EEMO ameliorated the impaired antioxidative defense system in rat livers, as indicated by the restoration of enzymatic activities (36,37). Hepatocytic damage is also characterized by different hepatic marker enzymes like ALT, AST and ALP. When liver cells are damaged, these enzymes leak into the bloodstream from liver tissue and produce markedly elevated serum levels. Here our work shows that CCl_4 administration causes severe acute liver damage in rats which is characterised by hepatic cell necrosis, ballooning degeneration, fatty changes or inflammatory cell infiltration (38). The reduction in activities of the biochemical parameters after treatment with EEMO indicates the protective effect of EEMO on the liver abnormalities which was caused by CCl_4.

CONCLUSION

The present study demonstrated that the ethanolic extract obtained from the aerial parts of *Macrothelypteris ornata* (family Thelypteridaceae) possesses significant hepatoprotective activity against CCl_4 induced oxidative stress in Wistar albino rats. Acute toxicity studies on Swiss albino mice revealed that EEMO has a reasonable safety profile. The hepatoprotective activity of EEMO may be due to the presence of flavonoids.

The authors acknowledge Seven Hills College of Pharmacy and Global Vision Educational Society for providing necessary facilities to carry out this research work.

AUTHOR CONTRIBUTIONS

AG carried out the research work and drafted the manuscript. LY involved in conceptualization, designing and edited manuscript.

REFERENCES

1. Grant, D.M. (1991). Detoxification pathways in the liver. J Inherit Metab Dis, 14(4),421-30.
2. Trefts, E., Gannon, M., Wasserman, D.H. (2017).The liver. Curr Biol, 6, 27(21), R1147-R1151.
3. Sandermann, H. (1994) Higher plant metabolism of xenobiotics: the 'green liver' concept. Pharmacogenetics, 4(5), 225-41.
4. Massart, J., Begriche, K., Corlu, A., Fromenty, B. (2022). Xenobiotic-Induced Aggravation of Metabolic-Associated Fatty Liver Disease. Int J Mol Sci, 19, 23(3),1062.
5. Mohi-Ud-Din, R., Mir, R.H., Sawhney, G., Dar, M.A., Bhat, Z.A. (2019). Possible Pathways of Hepatotoxicity Caused by Chemical Agents. Curr Drug Metab, 20(11), 867-879.
6. Weber, L.W., Boll, M., Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol, 33(2),105-36.
7. Brattin, W.J., Glende, E.A. Jr., Recknagel, R.O. (1985) Pathological mechanisms in carbon tetrachloride hepatotoxicity. J Free Radic Biol Med. 1(1), 27-38.
8. Unsal V, Cicek M, Sabancilar İ. (2020): Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Rev Environ Health. 36(2):279-295.
9. Vun-Sang S, Rodrigues KF, Dsouza UJA, Iqbal M. (2022): Suppression of Oxidative Stress and Proinflammatory Cytokines Is a Potential Therapeutic Action of *Ficus lepicarpa* B. (Moraceae) against Carbon Tetrachloride (CCl_4)-Induced Hepatotoxicity in Rats. Molecules. 18;27(8):2593.
10. Nwidu LL, Oboma YI, Elmorsy E, Carter WG. (2018): Alleviation of carbon tetrachloride-induced hepatocellular damage and oxidative stress with a leaf extract of Glyphae brevis (Tiliaceae). J Basic Clin Physiol Pharmacol. ;29(6):609-619.
11. Jahan A, Shams S, Ali S, Samrana S, Ali A, Adhikari A, Sajid M, Ali A, Ali H. (2021): Govaniadine Ameliorates Oxidative Stress, Inflammation, and Kupffer Cell Activation in Carbon Tetrachloride-Induced Hepatotoxicity in Rats. ACS Omega.6(4):2462-2472.
12. Kilany OE, El-Beltagy MA, El-Sherbeeny NA. (2020): Tribulus terrestris ameliorates carbon tetrachloride-induced hepatotoxicity in male rats through suppression of oxidative stress and inflammation. Environ Sci Pollut Res Int. 7(20):24967-24981.
13. Hussain S, Asrar M, Rasul A, Sultana S, Saleem U. (2022): *Chenopodium album* extract ameliorates carbon tetrachloride induced hepatotoxicity in rat model. Saudi J Biol Sci. 29(5):3408-3413.
14. Uday KS, Bhakat RK. (2021): Assessment of Pteridophytes' composition and conservation status in sacred groves of Jhargram District, South West Bengal, India. Biodiversitas ;22(5): 3171-3178.
15. Sander LC. (2017): Soxhlet Extractions. J Res Natl Inst Stand Technol. Jan 12;122:1.
16. Abubakar AR, Haque M. (2020): Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci.;12(1):1-10.
17. Abbasi WM, Ahmad S, Perveen S, Rehman T. (2017): Preliminary phytochemical analysis and in vivo evaluation of antipyretic effects of hydro-methanolic extract of *Cleomescaposa* leaves. J Tradit Complement Med. 29;8(1):147-149.
18. Kumbhar ST, Patil SP, Une HD. (2018): Phytochemical analysis of *Canna indica* L. roots and rhizomes extract. Biochem Biophys Rep. Oct 5;16:50-55.
19. Sokomba E, Wambebe C, Chowdhury BK, Iriah J, Ogbeide ON, Orkor D. (1986): Preliminary phytochemical, pharmacological and antibacterial studies of the alkaloidal extracts of the leaves of *Synclisia scabrida* Miers. J Ethnopharmacol. 1986 Nov;18(2):173-85.
20. Diener W., Mischke U., Schlede E., Kayser D. (1995): The biometric evaluation of the OECD modified version of the acute toxic category method (oral) Arch. Toxicol. 69:729–734.
21. OECD (2002), *Test No. 423: Acute Oral toxicity - Acute Toxic Class Method*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264071001-en.
22. Janakat S, Al-Merie H. (2002): Optimization of the dose and route of injection, and characterisation of the time course of carbon tetrachloride-induced hepatotoxicity in the rat. J Pharmacol Toxicol Methods. 2002 Jul-Aug;48(1):41-4.
23. Hwang YP, Choi CY, Chung YC, Jeon SS, Jeong HG. (2007): Protective effects of puerarin on carbon tetrachloride-induced hepatotoxicity. Arch Pharm Res. 2007 Oct;30(10):1309-17.
24. Awaad AS, Soliman GA, El-Sayed DF, El-Gindi OD, Alqasoumi SI. (2012): Hepatoprotective activity of *Cyperus alternifolius* on carbon tetrachloride-induced hepatotoxicity in rats. Pharm Biol. 50(2):155-61.
25. Deniz GY, Laloglu E, Koc K, Geyikoglu F. (2019): Hepatoprotective potential of *Ferula communis* extract for carbon tetrachloride induced hepatotoxicity and oxidative damage in rats. Biotech Histochem. 94(5):334-340.

26. Okhawa H, Ohishi N, Yagi K. (1979): Assay for lipid peroxides in animals and tissue by thiobarbituric acid reaction. Anal Biochem 95(2):351-358.
27. Ellman GL. (1959): Tissue Sulfhydryl groups. Arch Biochem Biophy; 82(1):70-77.
28. Sinha KA. (1972): Colorimetric assay of catalase. Anal Biochem;47: 389–94.
29. Shaban NZ, Aboelsaad AM, Awad D, Abdulmalek SA, Shaban SY. (2022): Therapeutic effect of dithiophenolato chitosan nanocomposites against carbon tetrachloride-induced hepatotoxicity in rats. Environ Sci Pollut Res Int. 29(6):8487-8502.
30. Hassan MH, Edfawy M, Mansour A, Hamed AA. (2012): Antioxidant and antiapoptotic effects of capsaicin against carbon tetrachloride-induced hepatotoxicity in rats. Toxicol Ind Health. 28(5):428-38.
31. El-Newary SA, Ismail RF, Shaffie NM, Hendawy SF, Omer E, Ahmed MM, ELsayed WM. (2021): Hepatoprotective effects of *Tagetes lucida* root extract in carbon tetrachloride-induced hepatotoxicity in Wistar albino rats through amelioration of oxidative stress. Pharm Biol. 59(1):986-997.
32. Ingawale DK, Mandlik SK, Naik SR. (2014): Models of hepatotoxicity and the underlying cellular, biochemical and immunological mechanism(s): a critical discussion. Environ Toxicol Pharmacol. 37(1):118-33.
33. Jia R, Cao LP, Du JL, Wang JH, Liu YJ, Jeney G, Xu P, Yin GJ. (2014): Effects of carbon tetrachloride on oxidative stress, inflammatory response and hepatocyte apoptosis in common carp (*Cyprinus carpio*). Aquat Toxicol.152:11-9.
34. Basu S. (2003): Carbon tetrachloride-induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. Toxicology. 189(1-2):113-27.
35. Tian Z, Jia H, Jin Y, Wang M, Kou J, Wang C, Rong X, Xie X, Han G, Pang X. (2019): Chrysanthemum extract attenuates hepatotoxicity via inhibiting oxidative stress *in vivo* and *in vitro*. Food Nutr Res. 2019 Apr 15;63.
36. Wu ZM, Wen T, Tan YF, Liu Y, Ren F, Wu H. (2007): Effects of salvianolic acid a on oxidative stress and liver injury induced by carbon tetrachloride in rats. Basic Clin Pharmacol Toxicol. 2007 Feb;100(2):115-20.
37. Ibrahim ZS, Nassan MA, Soliman MM. (2016): Ameliorative effects of pomegranate on carbon tetrachloride hepatotoxicity in rats: A molecular and histopathological study. Mol Med Rep. 13(4):3653-60.
38. Alshammari GM, Balakrishnan A, Chinnasamy T. (2017): 2-Hydroxy-4-methoxy benzoic acid attenuates the carbon tetra chloride-induced hepatotoxicity and its lipid abnormalities in rats via anti-inflammatory and antioxidant mechanism. Inflamm Res. 66(9):753-763.