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Carbon Tetra Chloride Induced Hepatoprotective Activity of Methanolic Extract of *Euphorbia thymofolia* Linn Using Albino Rats

Papiya Ghosh*, Vijeta Rajoriya, Alok Pal Jain
RKDF Collage of Pharmacy, NH-12, Hoshangabad Road , Bhopal (M.P.)

*Corresponding Author's E mail: papiyaghosh.279@gmail.com

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ABSTRACT

Methanolic extract of *Euphorbia thymifolia* showed the presence of Alkaloids, Carbohydrates, Glycosides, Phytosterols and Triterpenoids, Phenolic and Tannins, flavonoids and Saponins Hepatoprotective activity of MEET on CCl4- treated rats are shown in Table 2. It is well documented that CCl4 is biotransformed under the action of microsomal cytochrome P-450 of liver to reactive metabolites. These metabolites attributed to damage structural integrity of liver and raise the levels of SGPT. In the present study, SGPT level of positive control, Negative control, standard and different dose of plant extracts. Methanolic extract of the whole plant of *E. thymifolia* (MEET) at a dose of 100, 200 mg/kg, p.o. caused a significant inhibition in the levels SGPT towards the respective normal range is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage. The pretreatment with extract has prevented oxygen free radicals and thereby prevented the formation of peroxy radicals. This aspect of test extract also contributes to the hepatoprotectivity. From the results of the present investigation, it may be concluded that the aqueous extract of the leaves parts of *Euphorbia thymifolia* possess significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity.

K**eywords:** *Euphorbia thymifolia,* Hepatoprotective, CCL_4, Phytochemical analysis

INTRODUCTION

Impairment of vital organs like liver leads to serious consequences for the health of an individual and, in the majority of cases, is life threatening. Management of liver diseases is still a challenge to the modern medicine.[1] Modern medicines have little role to alleviation of hepatic diseases and the plant-based preparations which are chiefly available medicines employed for the treatment of liver disorders. The effectiveness of these plant products must be proved so as to identify newer medicaments acting against hepatic injury. In the absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal plants in Ayurveda are recommended for the treatment of liver disorders. Natural treatments from medicinal plants are considered

to be effective and safe medicaments for hepatotoxicity.[2] CCl4 is commonly used for free radical induced liver injury.[3] Liver is not the only target organ of CCl4 but it also affects several organs of the body such as lungs, hearts, testes, kidneys and brain.[4] It was reported from the investigation carried out on animal models of acute CCl4 induced liver damage, It is now generally accepted that CCl4 toxicity results from bioactivation of CCl4 into trichloromethyl free radical by cytochrome P450 system in liver microsomes and consequently causes lipid peroxidation of membranes that leads to liver.[5]

Euphorbia thymifolia is commonly known as *laghududhika* or *choti-dudhi*.[6,7] Over half a century after launching therapy for treatments, phytochemicals have become an important part of drugs. Actually, 70% of drugs approved between 1940 and 2002 are either natural products or have been developed based on knowledge gained from natural products.[8] But still, no scientific investigation has so far been reported in the literature regarding its action on the liver. Therefore, the present study was aimed at evaluating the hepatoprotective activity of *Euphorbia thymifolia* leaves on rat liver damage induced by CCL_4.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade and purchased from Himedia Lab Limited, India. Biochemical estimations were carried out using kits purchased from Ecoline Merck Limited, India.

Plant collection and extraction

The leaves of *Euphorbia thymifolia* were collected from in and around Bhopal District, Madhya Pradesh. The leaves of the plant *Euphorbia thymifolia* were dried in the shade, milled into coarse powder by a mechanical grinder and about 250gm of dry powder packed into Soxhlet apparatus and extracted with methanol at 45-50°C for 72hrs. The extract obtained was evaporated at 45°C, then dried and stored in airtight container. Finally the percentage yields were calculated of the dried extracts. The dried extract subjected for phytochemical analysis using standard methods [9].

Acute toxicity study

This study was carried out as per OECD test guideline 423 (OECD, 2001) in Wistar albino rats. The Animal Ethics Committee of the institution approved the study protocol. The extract fell under class 4 (LD50 > 2000 mg/kg). One-tenth and one-fifth of this dose was selected as the therapeutic dose for the evaluation of hepatoprotective activity [10].

Experimental animals

Male/female albino Wistar rats weighing 200 ± 20 gm used in this study were obtained from the Animal House of college. The animals were maintained under standard laboratory conditions of constant temperature (24 ±

2^0C), relative humidity (50% ± 15%), 12 h light: 12 h dark cycle, and allowed free access to food and water. Animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland and approved by the Committee for Animal Care at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA).

Experimental design

In the present study animals were divided into five main groups with six rats in each group:

Group 1 - Positive control (Vehicle treated) Group 2 - Negative control (Disease induced) Group 3 - Standard (Silymarin)

Group 4 - Test Group I (Methanol extract 100mg/kg)

Group 5 - Test Group II (Methanol extract 200mg/kg)

The study was performed by using Sprague Wistar male & female rats were divided into various groups, each group had six animals. Hepatotoxicity was induced using CCl4 (0.5 ml/kg, s.c.) for 9 days. On the 10th day, all the animals were sacrificed under anesthesia and blood as well as liver samples were collected for biochemical and histopathological investigation. For histopathological studies, liver sections were prepared, stained with alum hematoxylin and eosin, examined microscopically for histopathological changes.

Statistical analysis

Values are expressed in mean ± SD for six rats in each group. P value was calculated using one way ANOVA followed by Newmann Keul's multiple range tests. Values of $p<0.01$ were considered significant in all cases.

RESULTS AND DISCUSSIONS

The crude extract so obtained after the extraction process, extract was further concentrated on water bath evaporation the solvent completely to obtain the actual yield of extraction. The results of qualitative phytochemical analysis of the crude powder of leaves of *Euphorbia thymifolia* are shown in Table 1. Methanolic extract of *Euphorbia thymifolia* showed the presence of Alkaloids, Carbohydrates, Glycosides, Phytosterols and Triterpenoids, Phenolic and Tannins, flavonoids and Saponins Table 1.

Table 1 Phytochemical tests of Methanolic extract of *Euphorbia thymifolia* Linn

S. No.	Chemical Test	Inference
1.	**Tests for Alkaloids**	
	Mayer's reagent:	Positive
	Dragendroff's reagent:	Positive
	Wagner's reagent	Positive
	Hager's reagent	Positive
2.	**Tests for carbohydrates**	
	Molisch's test	Positive
	Barfoed's test	Positive
	Fehling's test	Positive
	Benedict's test	Positive
3.	**Tests for Glycosides**:	
	Modified Borntrager's test	Positive
	Keller killiani's test	Positive
	Legal test	Positive
	Baljet test	Positive
4.	**Tests for Protein**	
	Millon's test	Negative
5.	**Amino acids:**	
	Ninhydrin test	Negative
6.	**Tests for phytosterols and Triterpenoids**	
	Liebermann's Test	Positive
	Libermann-Burchard test	Positive
	Salkowaski Test	Negative
7.	**Test for Phenolic and Tannins**	
	Ferric Chloride Test	Positive
	Lead acetate Test	Positive
8.	**Test for flavonoids**	
	Shinoda's test	Positive
	Alkaline reagent test	Positive
9.	**Tests for Saponins**	
	Foam Test	Positive

Hepatoprotective activity of MEET on CCl4- treated rats are shown in Table 2. It is well documented that CCl4 is biotransformed under the action of microsomal cytochrome P-450 of liver to reactive metabolites. These metabolites attributed to damage structural integrity of liver and raise the levels of SGPT. In the present study, SGPT level of positive control, Negative control, standard and different dose of plant extracts. Methanolic extract of the whole plant of E. thymifolia (MEET) at a dose of 100, 200 mg/kg, p.o. caused a significant inhibition in the levels SGPT towards the respective normal range is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage.

Thus, the present investigation revealed hepatic enzymes SGPT, in serum significantly ($P < 0.001$) increased in CCl4 treated animals when compared to control. The MEET treatment (100 mg/kg) did not have significant effect on the levels of hepatic enzymes when compared to CCl4-treated animals. The MEET treatments (200 mg/kg) significantly ($P < 0.05$, $P < 0.01$; respectively) reversed the levels of hepatic enzymes when compared to CCl4- treated animals. Silymarin (25 mg/kg)-treated animals also showed significant ($P < 0.01$) inverted the levels of hepatic enzymes when compared to CCl4-treated animals as compared to the control group, which was significantly ($P <$

0.01 and $P < 0.05$) reversed with the treatment of MEET (200 mg/kg) and MEET (100 mg/kg), respectively.

Table 2. Effect of Euphorbia thymifolia Linn extract on serum liver enzymes (ALT,AST, and ALP), total protein in CCl4 induced liver damage in rats

S. No.	Group	SGOT	SGPT	ALP	Protein
1	Positive	25.9±3.55	26.5±3.45	864.49 ± 2.388	11.55±0.75
2	Negative	145.4±10.61*	450.5±15.38*	261.36 ± 3.189	3.30±045
3	Standard	79.5±0.33***	350±15.38***	98.3 ± 1.247***	7.55±0.35***
4	Test (100mg/kg)	99±8.25*	260±10.25*	222.3 ± 5.456*	6.95±0.35**
5	Test 200(mg/kg)	85.5±7.52***	210±12.24***	144.9 ± 13.358***	8.56±0.45***

Histopathological Studies

After the required amount of liver tissues were utilised for homogenate preparation, the remaining liver tissues were washed with normal saline and fixed in 10% formalin. Paraffin sections were prepared and stained with haematoxylin; eosin thereby examined using light microscopy (x100 magnification). Livers were removed from the experimental mice, cut into small pieces and fixed in 10% formaldehyde solution for overnight followed by dehydration. Dehydrated tissues were embedded in paraffin. 4µm sections were cut using microtome. Then liver sections were dewaxed in xylene, rehydrated in a series of different grades of alcohol and then washed with distilled water for 5 min. The liver sections were stained with basic stain haematoxylin for 40 sec and counter stained with acidic stain eosin for 20 sec. After proper staining the slides were observed (100X and 400X) using Nikon ECLIPSE 200 microscope figure 1.

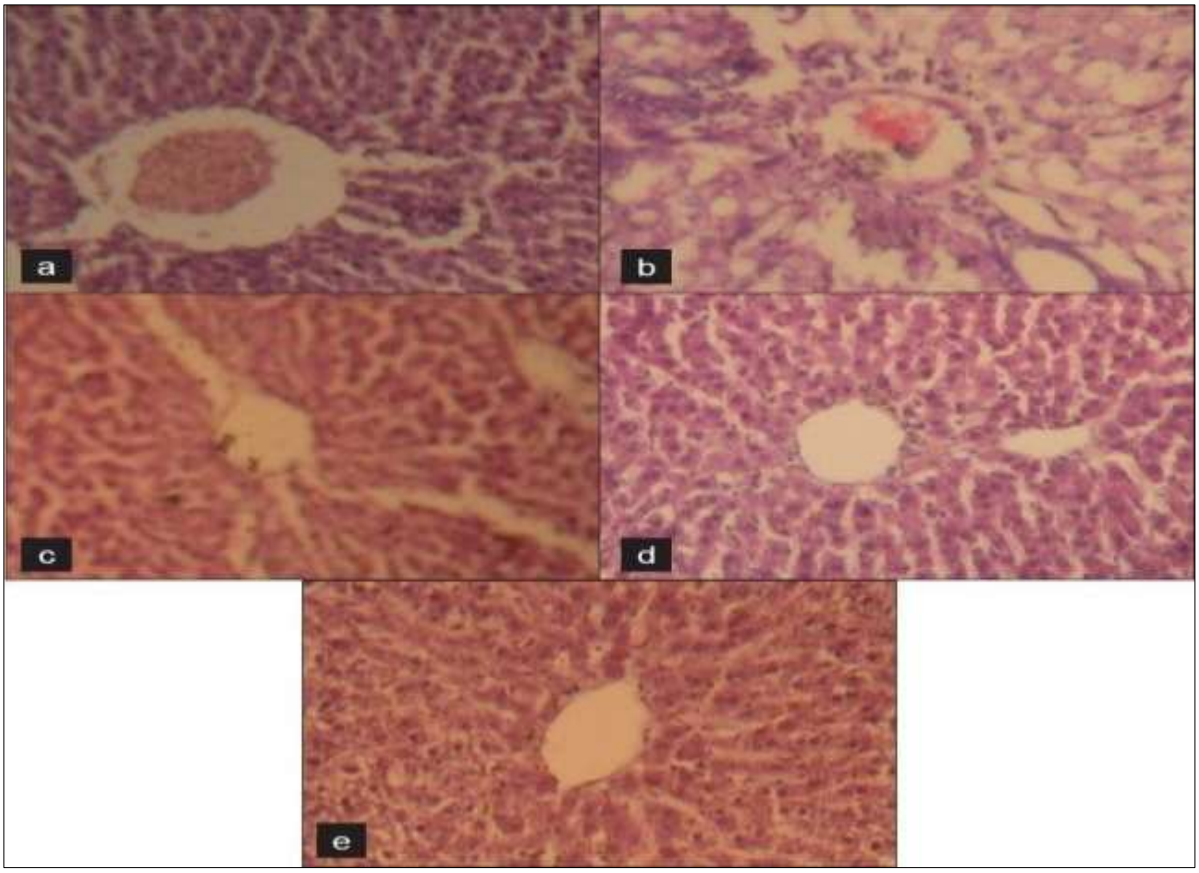

Figure 1. Histopathological study of liver tissue in control, , silymarin, and *Euphorbia thymifolia* Linn extract groups of rats. (a) Control group showed normal liver architecture (H&E ×40). (b) negative control group showed marked hepatic cell necrosis and moderate inflammation and lymphocytic infiltrations (H&E ×40). (c) Silymarine showed nearly normal liver structure(H&E ×100). (d) *Euphorbia thymifolia* Linn *extract* (100mg/kg) showed necrosis (H&E ×100).(e) *Euphorbia thymifolia* Linn *extract* (200mg/kg) showed mild necrosis and inflammation (H&E ×100).

CONCLUSION

The pretreatment with extract has prevented oxygen free radicals and thereby prevented the formation of peroxy radicals. This aspect of test extract also contributes to the hepatoprotectivity. From the results of the present investigation, it may be concluded that the aqueous extract of the leaves parts of *Euphorbia thymifolia* possess significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity.

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