HEPATOPROTECTIVE EFFECT OF EUPHORBIA THYMIFOLIA WHOLE PLANT EXTRACT ON CCl₄ INDUCED HEPATIC DAMAGE IN RATS
Syed Aamir¹*, Thippleswamy B S², Kulkarni V H³, Hegde Karunakar⁴
¹Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Post- Parangepete, Mangalore, Karnataka, India
²Department of Pharmacology, Siddaganga College of Pharmacy, Tumkur, Karnataka, India
³Department of Pharmacology, SET’s College of Pharmacy, Dharwad, Karnataka, India

Received on: 04/02/2011 Revised on: 12/03/2011 Accepted on: 22/03/2011

ABSTRACT
The present study has been designed to evaluate the hepatoprotective and in-vivo antioxidant activity of ethanolic extract of the whole plant of Euphorbia thymifolia (EEET) against carbon tetrachloride (CCl₄) - induced hepatotoxicity in experimental rats. The levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), uric acid, total protein and total bilirubin were determined. Antioxidant status in liver was determined by measuring the activity of malondialdehyde (MDA), glutathione (GSH) and catalase (CAT). Total wet weight and histopathological study of isolated liver specimen was also carried out. The oral pre-treatment with EEET (100, 200 mg/kg) showed significant hepatoprotection against CCl₄ induced hepatotoxicity by decreasing the activities of serum marker enzymes, bilirubin and lipid peroxidation, and significant increase in the levels of uric acid, GSH, CAT and protein in a dose dependent manner, which was confirmed by the histopathological examinations. Data also showed that EEET possessed strong antioxidant activity, which may probably leads to the promising hepatoprotective activities of Euphorbia thymifolia whole plant extract.

KEYWORDS: Antioxidants, Carbon tetrachloride, Euphorbia thymifolia, Hepatoprotective activity.

*Corresponding author
Syed Aamir, Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Post- Parangepete, Mangalore, Karnataka, India E-mail: aamirmaz@gmail.com

INTRODUCTION
Liver is an important organ that plays an essential role in regulating various physiological and metabolic processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has a great capacity to synthesize useful principles that regulate internal chemical environment. The main function of liver is metabolism and disposition of xenobiotic agents by exposing directly or indirectly. The xenobiotic agents such as carbon tetrachloride, paracetamol, nitrosamine and excess consumption of alcohol induce oxidative stress, which in turn damages liver cells. Liver is rich in antioxidant enzymes such as glutathione, superoxide dismutase and catalase that can metabolize reactive oxygen species. However, these reactive oxygen species (ROS) are also scavenged by the non enzymatic antioxidants like vitamin E, vitamin C and glutathione. Reactive oxygen species are well recognized in the pathogenesis of various diseases such as cancer, atherosclerosis, inflammation, diabetes, parkinsonism, etc. Thus, a balanced intake of antioxidant with scavenging action of ROS is important for prevention of the disease. Therefore the uses of antioxidants, both natural and synthetic are gaining worldwide importance in the prevention of various diseases.

In spite of phenomenal growth of modern medicine, there are no synthetic drugs available for the liver disorders. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Therefore plants have proven to be used effectively as hepatoprotective agents. One such plant is Euphorbia thymifolia (Syn: Chamaesyce thymifolia Linn.) belonging to the family Euphorbiaceae, locally available has been used in traditional system of medicine. A small annual herb, divaricately branched up to 20cm in diameter, grayish green and usually reddish purple tinged on all parts. Whole plant is used for treating diarrhoea, dysentery,
painful bleeding piles, cough, skin disease, parasitic infection, promotes conception properties, aphrodisiac and age-sustaining properties. The expressed juice is administered internally with wine as a remedy for snake-bite, and it is applied externally to the part bitten. It was used by tribal healers of India to treat liver diseases. However, no scientific data are available to validate the folklore claim. Keeping the above information in view, the present study was designed to evaluate the hepatoprotective potential and in-vivo antioxidant role of the ethanolic extract of whole plant of *Euphorbia thymifolia* in CCl₄ induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Plant material and preparation of the extract**
The whole plant of *Euphorbia thymifolia* were collected in the month of April 2010 from the surrounding fields of Dharwad and authenticated by Dr. G S Hegde, Professor and Head, Department of Botany, Karnatak University, Dharwad. The shade dried whole plant was powdered by using grinder. The coarse powder was packed into Soxhlet column and extracted with 70% ethanol for 24 hrs. The solvent was evaporated to syrupy consistency using rotary flash evaporator to give ethanol extract (8% yield). The dried extract was stored in an airtight container in refrigerator until used. Suspension of the extract was prepared in 2% Tween-80 and used for hepatoprotective activity.

**Chemicals**
All the chemicals and solvents were of analytical grade and were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India. Carbon tetrachloride (CCl₄) was procured from E. Merck (India) Ltd, Mumbai. Silymarin was purchased from Micro Labs India Ltd, India. Standard kits for SGOT, SGPT, SALP, uric acid and bilirubin were obtained from Erba Diagnostics Mannheim GmbH Labs–Germany. The absorbance was measured using UV-Visible spectrophotometer (Jenway).

**Experimental animals**
The Wistar albino rats of either sex (150–200 g) were selected for experiment. The animals were procured from Shri Siddaganga College of Pharmacy, Tumkur, Karnataka. After randomization into various groups, animals were acclimatized for a period of 5 days under standard laboratory conditions (Room temperature 27±3°C, relative humidity 65±10%, 12 h light/dark cycle). All the animals were fed with standard rodent pellet (Amrut Feeds, Venkateshwara Enterprises, Bangalore) and water ad-libitum under strict hygienic condition. Ethical clearance for performing animal experiment was obtained from Institutional Animal Ethics Committee (IAEC) prior to the initiation of the experiment and the care of the laboratory animals was taken as per the CPCSEA regulations.

**Acute toxicity study**
The acute toxicity of 70% ethanolic extract of the whole plant of *Euphorbia thymifolia* (EEET) was determined in female albino rats. The animals were fasted overnight and the ethanolic extract was administered orally with a starting dose of 2000 mg/kg, to different groups of animals. Animals were observed continuously for first 3 hours and monitored for 14 days for any mortality or general behavior of animals, signs of discomfort and nervous manifestations. There was no mortality amongst the graded dose groups of animals and did not show any toxicity or behavioral changes at a dose level of 2000 mg/kg. This finding suggests that the EEET is safe in non-toxic to rats and hence doses of 100, 200 mg/kg, p.o. was selected for the study.

**Phytochemical screening**
Freshly prepared ethanol extract of whole plant of *Euphorbia thymifolia* was subjected to preliminary phytochemical screening for detection of major chemical constituents.

**CCl₄ induced hepatotoxicity**
Wistar albino rats were randomly divided in to six groups of six animals each. Group I, the normal control group was administered a single daily dose of normal saline (5 ml/kg, p.o.). Group II, the CCl₄ control group was administered a single daily dose of normal saline (5 ml/kg, p.o.) and CCl₄/Olive oil (1:1 v/v, 0.7 ml/kg, i.p.) on alternate days for a period of 7 days. Group VI, the standard group was administered a single daily dose of silymarin (100 mg/kg, p.o.) and CCl₄/Olive oil (1:1 v/v, 0.7 ml/kg, i.p.) on alternate days for a period of 7 days. Group III, the test group was administered a single daily dose of EEET 200 mg/kg, p.o. only, for a period of 7 days. Group IV and V, the test groups were administered a single daily dose of EEET (100 and 200 mg/kg, p.o., respectively) and CCl₄/Olive oil (1:1 v/v, 0.7 ml/kg, i.p.) on alternate days for a period of 7 days.

**Assessment of hepatoprotective activity**
All the animals were sacrificed 2 h after the administration of the drug on seventh day. Blood was collected by retro-orbital bleeding under mild ether anesthesia. Blood was allowed to clot at room temperature for 30 min, centrifuged at 3000 rpm for 15 min and subjected to estimate biochemical parameters. Liver was dissected and stored in 10% formalin solution for histopathological study.

**Determination of antioxidant activity**
From all the experimental groups, the portion of the liver was collected and rinsed with 0.15 M Tris-HCl (pH 7.4). A 10% w/v of liver homogenate was prepared in 0.15 M...
Tris-HCl buffer and processed for the estimation of lipid peroxidation in the form of malondialdehyde (MDA) in liver by measuring the thiobarbituric acid reactive substance (TBARS). A part of the homogenate after precipitating proteins with 20% trichloro acetic acid (TCA) containing 1 mM EDTA, the supernatant was used for reduced glutathione (GSH) estimation. The rest of the homogenate was centrifuged at 2000 rpm for 10 min at 4°C. The cell free supernatant thus obtained was used for the estimation of catalase (CAT) activity.

**Statistical analysis**
The data were expressed as the mean ± SEM, (n = 6). Data were analyzed using One way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post hoc test (SPSS 10.0 for Windows). Values of \( P < 0.05 \) were considered statistically significant.

**RESULTS**

**Phytochemical screening**

Preliminary phytochemical investigation of the EEET led to the presence of flavonoids, glycosides, terpenes, volatile oils, carbohydrates, triterpenoids and tannins.

**Effect of EEET on serum marker enzyme levels**

There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT and SALP content of CCl\(_4\) intoxicated animals. In contrast, pre-treatment with EEET (100, 200 p.o.) and silymarin (100 mg/kg, p.o.) exhibited an ability to counteract the hepatotoxicity by decreasing serum marker enzymes in a dose dependent manner (\( P < 0.05 \)) (Table 1).

**Effect of EEET on biochemical parameters**

In CCl\(_4\) treated groups, there was a significant increase in total bilirubin and significant reduction in uric acid and total protein content. Where as, pre-treatment with EEET (100, 200 mg/kg, p.o.) caused significant reduction in total bilirubin and significant increase in the activities of uric acid and total protein content dose dependently (Table 1).

**Effect of EEET on antioxidant activity**

The effect of EEET on rat liver lipid peroxidation (MDA), GSH and catalase levels were shown in Table 2. There was a significant increase in MDA content and reduction in GSH and catalase activities of CCl\(_4\) intoxicated animals. Pre-treatment with silymarin (100 mg/kg, p.o.) and EEET (100, 200 mg/kg, p.o.) significantly (\( P < 0.05 \)) prevented the increase in MDA levels and brought them near to normal level, where as GSH, and CAT levels were significantly raised, thus providing protection against CCl\(_4\) toxicities.

**Histopathology**

Histopathological studies also provided a supportive evidence for biochemical analysis. The liver sections of the rats with CCl\(_4\) intoxicated groups showed hepatic cells with severe toxicity characterized by inflammatory infiltration and necrosis in many areas. Pre-treatment with silymarin and EEET exhibited significant liver protection against CCl\(_4\) induced liver damage, which is evident by the presence of more or less normal hepatocytes and reduced inflammatory infiltration and necrosis (Fig. 1a – 1f).

**DISCUSSION**

Liver damage induced by CCl\(_4\) are commonly used model for the screening of hepatoprotective drugs. It is well documented that CCl\(_4\) 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, SGOT, SALP, bilirubin and uric acid. Further depletion of GSH, decreased protein synthesis, triglycerides accumulation, increased lipid peroxidation, destruction of Ca\(^{2+}\) homeostasis results in hepatocyte damage.

Hepatocellular necrosis or membrane damage leads to very high level of GOT, GPT and ALP to release from liver to circulation. The elevated levels of these serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver. It is well known that toxicants like CCl\(_4\) produces sufficient injury to hepatic parenchyma cells to cause elevation in serum bilirubin, in contrast decreases the level of total plasma protein content.

In the present study, ethanol extract of the whole plant of *E. thymifolia* (EEET) at a dose of 100, 200 mg/kg, p.o. caused a significant inhibition in the levels SGPT and SGOT towards the respective normal range is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage. On the other hand suppression of elevated SALP activities with concurrent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants. These results indicate that EEET preserved the structural integrity of the hepatocellular membrane and liver cell architecture damaged by CCl\(_4\) which was confirmed by histopathological examinations. The reduced level of uric acid in CCl\(_4\) induced hepatotoxicity probably due to the increased utilization of uric acid against increased production of the free radicals, which is a characteristic feature of cancer and tissue necrosis. The result from the present study suggested that altered uric acid level to near normal in EEET treated animals could be due to strong antioxidant property of the extract. Lipid peroxidation has been postulated to be the destructive process in liver injury due to CCl\(_4\). In the
present study, an elevation in the levels of MDA in liver of animals treated with both the toxicants was observed. The increase in MDA levels of liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Pre-treatment with EEET significantly reduced the levels of lipid peroxidation. Hence it may be possible that the mechanism of hepatoprotection by EEET is due to its antioxidant potentials.

Glutathione (GSH) is one of the most abundant naturally occurring tripeptide, non-enzymatic biological antioxidant present in liver. Its functions are concerned with the removal of free radicals such as H$_2$O$_2$ and superoxide radicals, maintenance of membrane protein, detoxification of foreign chemicals and biotransformation of drugs. In the present study the decreased level of GSH has been associated with an enhanced level of lipid peroxidation in CCl$_4$ intoxicated groups of rats. Pre-treated with EEET significantly increased the level of glutathione in a dose dependent manner. Thus EEET may act by inducing the detoxifying enzymes and these enzymes might detoxify the ROS following administration of toxicants.

Serum activities of catalase (CAT) are the most sensitive enzymatic index in liver injury caused by ROS and oxidative stress. Catalase is a haemoprotein; protect the cells from the accumulation of H$_2$O$_2$ by dismutating it to form H$_2$O and O$_2$. Therefore reduction in the activities of these enzymes may indicate the toxic effects of ROS produced by toxicants. In the present study, it was observed that pre-treatment with EEET caused a significant raise in hepatic CAT activities. This suggests that EEET can reduce ROS that might lessen the oxidative damage to the hepatocytes and improve the activities of the liver antioxidant enzymes, thus protects the liver from carbon tetrachloride.

Preliminary phytochemical screening of the ethanol extract of the whole of *E. thymifolia* (EEET) revealed the presence of flavonoids, glycosides, terpenes, volatile oils, carbohydrates, triterpenoids, phenolic compounds and tannins. However, flavonoids, triterpenoids are known to possess hepatoprotective activity in animals. It is worthwhile to isolate the bioactive principles which are responsible for these activities, which is in progress in our laboratory.

It can be concluded that the data obtained in the present study suggest that the ethanolic extract of the whole of *E. thymifolia* (EEET) has significant hepatoprotective and antioxidant activities on both CCl$_4$ induced hepatic damage in rats. These results reveal that the hepatoprotective effect of the ethanolic extract of the whole of *E. thymifolia* (EEET) may be due to its ability to block the bioactivation of toxicant and its potent antioxidant activity, and/or by scavenging the free radicals and inhibiting the lipid peroxidation.

**ACKNOWLEDGEMENT**

The authors are thankful to the authorities of A Shama Rao Foundation Mangalore, Karnataka, India and Soniya College of Pharmacy, Dharwad, Karnataka, India for providing required facilities.

**REFERENCES**


Table 1: Effect of ethanol extract of the whole plant of *Euphorbia thymifolia* Linn (EEET) on serum enzyme and biochemical parameters in CCl4 induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg, p.o.)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>SALP (U/L)</th>
<th>Uric acid (mg/dl)</th>
<th>Total Protein (mg/dl)</th>
<th>Total Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>5 ml</td>
<td>21.7 ± 1.0</td>
<td>21.7 ± 1.0</td>
<td>138.5 ± 12.3</td>
<td>0.9 ± 0.02</td>
<td>56.4 ± 2.1</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1.0 ml</td>
<td>143.6 ± 3.3</td>
<td>151.1 ± 6.2</td>
<td>331.3 ± 20.4</td>
<td>1.5 ± 0.03</td>
<td>16.2 ± 0.8</td>
<td>0.5 ± 0.04</td>
</tr>
<tr>
<td>EEET Alone</td>
<td>200</td>
<td>31.9 ± 1.8</td>
<td>21.6 ± 1.5</td>
<td>162.3 ± 14.8</td>
<td>1.1 ± 0.079</td>
<td>49.5 ± 4.3</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>CCl4 + EEET</td>
<td>100</td>
<td>121.3 ± 2.2*</td>
<td>110.0 ± 9.1*</td>
<td>243.5 ± 7.5*</td>
<td>1.2 ± 0.02*</td>
<td>28.9 ± 0.7*</td>
<td>0.3 ± 0.03*</td>
</tr>
<tr>
<td>CCl4 + EEET</td>
<td>200</td>
<td>98.4 ± 5.5**</td>
<td>49.5 ± 9.3***</td>
<td>212.3 ± 14.9**</td>
<td>1.0 ± 0.01**</td>
<td>40.5 ± 1.2**</td>
<td>0.2 ± 0.01**</td>
</tr>
<tr>
<td>CCl4 + Silymarin</td>
<td>100 mg/ml</td>
<td>104.1 ± 6.5***</td>
<td>68.1 ± 9.0***</td>
<td>188.0 ± 9.2***</td>
<td>0.9 ± 0.01***</td>
<td>49.0 ± 1.4***</td>
<td>0.2 ± 0.02***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 6, one way ANOVA followed by Tukey’s multiple comparison post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001 when compared with positive control group.

Table 2: Effect of ethanol extract of the whole plant of *Euphorbia thymifolia* Linn (EEET) on lipid peroxidation (LPO), glutathione (GSH), catalase (CAT) and liver weight in CCl4 induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg, p.o.)</th>
<th>LPO (mM MDA/mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>CAT (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>5 ml</td>
<td>2.9±0.3</td>
<td>21.3±0.5</td>
<td>88.0±1.3</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0.7 ml</td>
<td>19.9±1.2</td>
<td>6.20±0.5</td>
<td>10.8±0.8</td>
</tr>
<tr>
<td>EEET Alone</td>
<td>200</td>
<td>4.0±0.3</td>
<td>19.6±0.4</td>
<td>79.6±1.2</td>
</tr>
<tr>
<td>CCl4 + EEET</td>
<td>100</td>
<td>16.0±0.5*</td>
<td>9.2±0.3*</td>
<td>21.8±1.4*</td>
</tr>
<tr>
<td>CCl4 + EEET</td>
<td>200</td>
<td>11.1±0.6**</td>
<td>17.3±0.3**</td>
<td>59.7±3.1**</td>
</tr>
<tr>
<td>CCl4 + Silymarin</td>
<td>100</td>
<td>6.5±0.4***</td>
<td>20.1±0.6***</td>
<td>75.5±1.7***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 6, one way ANOVA followed by Tukey’s multiple comparison post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001 when compared with positive control group.

Fig. 1: Effect of ethanolic extract of the whole plant of *Euphorbia thymifolia* on CCl₄ induced liver damage in rats.

a. Negative control- showing normal architecture of hepatic cells, b. Positive control- showing inflammatory infiltration, fatty changes and necrosis, c. EEET 200mg alone treated group- showing normal architecture of hepatic cells no hepatic damage by test compound, d. Liver pretreated with EEET 100mg prior to CCl₄ administration- showing a pattern of reduced inflammatory infiltration and necrosis, e. Liver pretreated with EEET 200mg prior to CCl₄ administration- showing a pattern of reduced inflammatory infiltration and necrosis, f. Liver pretreated with silymarin prior to CCl₄ administration- showing normal architecture of hepatic cells with less fatty changes

Source of support: Nil, Conflict of interest: None Declared