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کارگاه آنلاین آشنا با یافته‌های اطلاعات علمی
پژوهشی و ترفندهای جستجو
Hepatoprotective and Antioxidant Activity of *Euphorbia tirucalli*

T.M. JYOTHI, M.M. SHANKARIAH, K. PRABHU, S. LAKSHMINARASU, G.M. SRINIVASA and SIDDAMSETTY SETTY RAMACHANDRA

For author affiliations, see end of text.

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**ABSTRACT**

Treatment of diseases with natural remedies is gaining popularity because of fewer side effects. A systemic and scientific investigation of aqueous extract of *Euphorbia tirucalli* for its antioxidant and hepatoprotective potential against carbon-tetrachloride-induced hepatic damage in rats was carried out. Antioxidant property was assessed by using reducing property, superoxide anion scavenging and hydroxyl radical scavenging property. Hepato-protective property was assessed by measuring the extent of reversal of enhanced biochemical markers of hepatitis, like serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase ALP, bilirubin, cholesterol, triglycerides and also by estimating the tissue glutathione (GSH) levels and the extent of reduction in the tissue lipid peroxidation. The aqueous extract has demonstrated dose-dependant invitro antioxidant property (at 20 μg, 40 μg, 60 μg, 80 μg, 100 μg) in all the models of the study. Similarly, aqueous extract of *Euphorbia tirucalli* at the doses of 125mg/kg and 250mg/kg produced significant hepatoprotective effect by decreasing the serum enzymes, bilirubin, cholesterol, triglycerides and tissue lipid peroxidation, while it significantly increased the levels of tissue GSH in a dose-dependant manner. From the present study, it may be concluded that the test extract possesses antioxidant and hepatoprotective properties. The hepatoprotective property may be attributed to its antioxidant potential.

**Keywords:** *Euphorbia tirucalli*, Antioxidant activity, Hepatoprotectivity, Carbon-tetrachloride

Hepatic system is very vital organ system involved in the body’s metabolic activities. As a result the chemical reactions in the liver may generate several reactive species like free radicals. These reactive species form covalent bond with the lipids of the tissue. However, built protective mechanisms combat the hazardous reactions associated with the free radicals. Due to excessive exposure to hazardous chemicals, the free radicals generated will be so high such that they overpower the natural defensive system leading to hepatic damage and cause jaundice, cirrhosis and fatty liver, which remain one of the serious health problems. Carbon tetra-chloride (CCl₄) is one such hazardous chemical which induces hepatopathy through membrane lipid peroxidation by its free radical derivative, (CCl₃·, CCl₂O₂·). Excessive production of the reactive species manifests in tissue-thiol depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injury [1]. In the background of the above, it is realized that antioxidant activity or inhibition of generation of free radicals plays a crucial role in providing protection against such hepatic damage.

Several herbs and herbal products are known to possess antioxidant principles and may be used as organ protective agents. Herbs belonging to Euphorbiaceae are reported have antioxidant principles like flavonoids and shown organ protective properties [2, 3]. *Euphorbia tirucalli* is a small tree easily recognized from the erect branches and smooth, terete, polished, whorled or fascicled branchlets. The juice is purgative, carminative; useful in gonorrhoea, whooping cough, asthma, dropsy, leprosy, enlargement of spleen, dyspepsia, jaundice, stone in the bladder [4]. The milky juice, is applied to stitch and scorpion bites, it is also a warm rubefacient remedy in rheumatism and toothache [5]. Isolated compounds from the plant include cycloeuphorin, euphorbol and n-hexacosanol [6]. Preliminary phytochemical investigation showed the presence of triterpenes & flavonoids, both of which are reported to possess hepatoprotective and antioxidant activity [7,8]. Similarly there
were claims from a local native practitioner that the plant ... in porcine. In addition to these claims, the plant has also been used in traditional medicine to treat a variety of conditions such as fever, inflammation, and dermatological issues. The bark of the plant is generally used as a diuretic, while the leaves are used as a remedial agent for fever.

The bark of ET was collected from the out fields of Harapanahalli, India in the month of September/October, and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, SCS College of Pharmacy. A voucher specimen is currently deposited in the Department of Pharmacognosy (SCSCP – PH – 01/2007).

**Extract preparation**

Aerial parts of ET were shade dried, ground to a coarse powder and subjected to successive extraction by using different solvents in the increasing order of their polarity (pet ether, chloroform and methanol) in soxhlet apparatus, until the eluent became colorless and then macerated with chloroform water [9]. The aqueous extract was dried under reduced pressure at a yield of 9.3% (w/w). From this extract, on evaporation of water in vacuum, a brown colored substance was obtained which was kept at 4°C until use.

**Preliminary phytochemical investigation**

All the extracts were subjected to preliminary phytochemical tests [9]. All the tests reveal that the plant possesses steroids, glycosides, triterpenoids, tannins and flavonoids. Since aqueous extract has shown the better results for the presence of polyphenolic compounds and triterpenoids, this extract was selected for further study.

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**Animals**

Adult Wistar rats (180-220 g) and Swiss albino mice (20-25 g) were used in this study. They were housed in well-ventilated rooms under standard conditions (23 ± 2°C, humidity 65-70%, 12 h light/dark cycle), fed with standard rodent pellet diet (Lipton India Ltd. Mumbai) and with tap water ad libitum. Permission was obtained from institutional ethical committee for the use of animals in experiments.

**Reducing power**

The reducing power was determined according to the method of Oyaizu [10]. Different doses of ET extract (20-100μg) were mixed in 1ml of distilled water (pH 7.4) and incubated at 50°C for 20 minutes. A porcine muscle homogenate was added to the mixture and animals were sacrificed by cervical decapitation and which was then centrifuged at 3000rpm for 10 minutes. Heparinized blood sample
were taken and assessed for serum enzyme markers and hepatic tissue was taken and subjected to histopathological study and further tissue was analyzed for Glutathione and lipid peroxidation.

Serum enzymes, which were assessed, include Serum glutamic oxaloacetate transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) [14], total bilirubin and direct bilirubin [15], cholesterol, triglycerides and alkaline phosphate (ALP) contents.

Table 1. Reducing power of aqueous extract of aerial parts of ET doses & SMS (n=3) and superoxide anion scavenging activity by PMS/NaOH-NBT method.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (µg)</th>
<th>Reducing Property (Abs)</th>
<th>Increase (%)</th>
<th>PMS-NaOH System (Abs)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.229 ± 0.0012</td>
<td>-</td>
<td>0.863 ± 0.0076</td>
<td>-</td>
</tr>
<tr>
<td>SMS</td>
<td>25</td>
<td>0.417 ± 0.0105*</td>
<td>82</td>
<td>0.121 ± 0.0012*</td>
<td>86</td>
</tr>
<tr>
<td>ET</td>
<td>20</td>
<td>0.271 ± 0.0241*</td>
<td>16</td>
<td>0.679 ± 0.0043*</td>
<td>21</td>
</tr>
<tr>
<td>ET</td>
<td>40</td>
<td>0.284 ± 0.0019*</td>
<td>20</td>
<td>0.612 ± 0.0127*</td>
<td>29</td>
</tr>
<tr>
<td>ET</td>
<td>60</td>
<td>0.318 ± 0.0312*</td>
<td>28</td>
<td>0.564 ± 0.0987*</td>
<td>35</td>
</tr>
<tr>
<td>ET</td>
<td>80</td>
<td>0.349 ± 0.0116*</td>
<td>52</td>
<td>0.491 ± 0.0030*</td>
<td>43</td>
</tr>
<tr>
<td>ET</td>
<td>100</td>
<td>0.373 ± 0.0053*</td>
<td>63</td>
<td>0.401 ± 0.0310*</td>
<td>54</td>
</tr>
</tbody>
</table>

* P-Value <0.001 Vs control group, Bonferroni test.
SMS → Sodium metabisulphate

Table 2. Hydroxyl radical scavenging activity of aqueous ext of aerial parts of ET by 2-deoxyribose degradation assy.

<table>
<thead>
<tr>
<th>Incubation system</th>
<th>Hydroxyl Determination By 2-deoxyribose degradation Assay (Abs 532 nm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abs Inhibition (%)</td>
<td></td>
</tr>
<tr>
<td>2-deoxyribose/phenylhydrazine</td>
<td>0.448±0.0035</td>
<td>-</td>
</tr>
<tr>
<td>2-deoxyribose/phenylhydrazine /SMS 25µg</td>
<td>0.088±0.0076*</td>
<td>80</td>
</tr>
<tr>
<td>2-deoxyribose/phenylhydrazine /ET 20µg</td>
<td>0.359±0.0065*</td>
<td>20</td>
</tr>
<tr>
<td>2-deoxyribose/phenylhydrazine /ET 40µg</td>
<td>0.312±0.0013*</td>
<td>30</td>
</tr>
<tr>
<td>2-deoxyribose/phenylhydrazine /ET 60µg</td>
<td>0.263±0.0065*</td>
<td>41</td>
</tr>
<tr>
<td>2-deoxyribose/phenylhydrazine /ET 80µg</td>
<td>0.208±0.0101*</td>
<td>54</td>
</tr>
<tr>
<td>2-deoxyribose/phenylhydrazine /ET 100µg</td>
<td>0.164±0.0276*</td>
<td>63</td>
</tr>
</tbody>
</table>

*, P-Value <0.001 Vs 2-deoxyribose/phenylhydrazine treated, Bonferroni test.
SMS → Sodium metabisulphate

Table 3. Effect of Aqueous extract of the aerial parts of ET in carbon tetrachloride-induced hepatotoxicity in rats (n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT(U/L) Mean ± SE</th>
<th>SGPT(U/L) Mean ± SE</th>
<th>ALP (U/L) Mean ± SE</th>
<th>Cholesterol (mg/dl) Mean ± SE</th>
<th>Triglycerides (mg/dl) Mean ± SE</th>
<th>Total Bilirubin (mg/dl) Mean ± SE</th>
<th>Direct Bilirubin (mg/dl) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>102 ± 5.48</td>
<td>51 ± 1.87</td>
<td>198±3.59</td>
<td>111±2.21</td>
<td>221±7.88</td>
<td>0.923±0.012</td>
<td>0.235±0.010</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>355 ± 4.95</td>
<td>167±3.69</td>
<td>1036±6.32</td>
<td>179±2.27</td>
<td>766±4.57</td>
<td>5.036±0.035</td>
<td>1.84±0.056</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>183 ± 4.93*</td>
<td>88±2.46*</td>
<td>483±2.52*</td>
<td>121±1.69*</td>
<td>463±2.03*</td>
<td>1.838±0.026*</td>
<td>0.248±0.006*</td>
</tr>
<tr>
<td>GROUP 4</td>
<td>284±2.822*</td>
<td>160±1.778*</td>
<td>951±4.906*</td>
<td>155±1.838*</td>
<td>699±3.149*</td>
<td>4.149±0.020*</td>
<td>0.713±0.005*</td>
</tr>
<tr>
<td>GROUP 5</td>
<td>213±2.671*</td>
<td>129±6.93*</td>
<td>712±2.777*</td>
<td>142±2.362*</td>
<td>606±2.156*</td>
<td>3.002±0.030*</td>
<td>0.553±0.002*</td>
</tr>
</tbody>
</table>

Values are the Mean ± SEM of six rats/treatment.
* P=0.001 Vs CCl4 treated group (group 2), Bonferroni test.
Group 1-Normal animals (Olive oil 1ml/kg,p.o)
Group 2-CCl4 (1ml/kg BW) treated animals
Group 3-CCl4 + Silymarin (100mg/kg BW,p.o.) treated animals.
Group 4-CCl4 + ET (125mg/kg BW, p.o.) treated animals.
Group 5-CCl4 + ET (250mg/kg BW, p.o.) treated animals.
Table 4. Effect of aqueous extract of aerial parts of ET on tissue GSH level and tissue lipid peroxidation (n=6) in carbon tetrachloride-induced hepatotoxicity in rats (n=6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/Kg BW)</th>
<th>GSH (Abs)</th>
<th>Increase (%)</th>
<th>Lipid Peroxidation (Abs )</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>-</td>
<td>0.799±0.001</td>
<td>-</td>
<td>0.099±0.001</td>
<td>-</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>-</td>
<td>0.239±0.015</td>
<td>-</td>
<td>0.292±0.008</td>
<td>-</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>100</td>
<td>0.445±0.006</td>
<td>86</td>
<td>0.088±0.003</td>
<td>70*</td>
</tr>
<tr>
<td>GROUP 4</td>
<td>125</td>
<td>0.292±0.002*</td>
<td>22</td>
<td>0.224±0.006*</td>
<td>23*</td>
</tr>
<tr>
<td>GROUP 5</td>
<td>250</td>
<td>0.387±0.002*</td>
<td>62</td>
<td>0.147±0.002*</td>
<td>50*</td>
</tr>
</tbody>
</table>

*a. P – Value <0.001 Vs normal saline – CCl4 treated, Bonferrni test.

B. P – Value <0.01 Vs normal saline – CCl4 treated, Bonferrni test.

Group 1-Normal animals (Olive oil1ml/kg,p.o)
Group 2-CCl4 (1ml/kg BW) treated animals
Group 3-CCl4 + Silymarin (100mg/kg BW,p.o) treated animals.
Group 4-CCl4 + ET (125mg/kg BW, p.o.) treated animals.
Group 5-CCl4 + ET (250mg/kg BW, p.o.) treated animals.

% Sodium nitrate) was added and the absorbance at 412 nm was measured immediately after mixing.

Extent of lipid peroxidation was done by combining TBA reactive material significantly over period 1 hour

1.0ml of biological sample (0.1 – 2.0 mg of membrane

0.1 ml of protein or 0.1 – 0.2 μmol of lipid phosphate) with 2.0

ml of TCA-TBA-HCl and thoroughly. The solution is

heated for 15min in a boiling water bath. After cooling,

the flocculent precipitate was removed by centrifugation

at 1000rpm for 10min. The absorbance of the sample

was determined at 535nm against blank that contains all

the reagents minus the lipid [18].

A remarkable elevation was observed in Serum

GOT, GPT, ALP, cholesterol, triglycerides, total Bilirubin and direct

bilirubin values in control (saline + vehicle) group of
rats are tabulated in table 3.

The estimated values of serum GOT, GPT, ALP, cholesterol, triglycerides, total Bilirubin and direct bilirubin values in CCl4 intoxicated

treated animals.

The tissue GSH depletion was inhibited by the pretreatment with test extract in a dose
dependent manner. Similarly lipid peroxidation induced

by CCl4 treatment was reversed by test extract in a dose
dependent manner. The results are compiled in table 4.

Aqueous extract was taken for assessing the

tissue GSH level and tissue lipid peroxidation (n=6) in carbon tetrachloride-induced hepatotoxicity in rats (n=6).

Histopathological reports show a promising response

on treatment with aqueous extract of aerial parts of ET

in rats (Toxic Control group). In the groups treated with

125mg/kg and 250mg/kg of the extract, the above bio-

chemical markers of hepatotoxicity were found to be
decreased when compared to CCl4 treated control group.

Evidently, the hepatoprotective effects of higher dose of

ET (250mg/kg) were near to that of standard i.e. Sily-

marin (100mg/kg). Both the doses of ET used in the

study showed significant protective property than con-

trol. However the test extract was found to be less po-

tent than that of standard drug.

The tissue glutathione was found to be depleted

upon CCl4 intoxication, indicate that the tissue damage

is due to over powering the inbuilt free radical scavenger

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reversed by the test extract and also reduced the extent of lipid peroxidation. Most of the mammals have an effective mechanism to prevent and neutralize the free radical induced damage, which is accomplished by a set of endogenous substances such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. In present study in vitro antioxidant activities showed significant increase in the absorption in reducing power and reduction in absorption in hydroxyl ion and superoxide anion scavenging activities, indicating that the study plant possesses antioxidant activities. In biochemical system, superoxide radical and H\textsubscript{2}O\textsubscript{2} react together to form the hydroxyl radical, this can attack and destroy almost all known biochemicals [19]. The hydroxyl radicals thus produced may attack the sugar of DNA base causing sugar fragmentation, base loss and DNA strand breaks, which is accomplished by a set of endogenous substances [13]. ET extract reduced the superoxide anions and also scavenge off the hydroxyl radicals and hence inhibit the cellular damage. It is apparent from the present study that the test extract do not interfere with the generation of the free radicals but it scavenge off the free radicals.

Fig 1. HISTOPATHOLOGY REPORT IN CCl\textsubscript{4} INDUCED HEPATOTOXICITY: Negative Control: Showed normal lobular architecture and normal hepatic cells with a well preserved cytoplasm and well-defined nuclei, nucleoli. Positive Control: Showed centrilobular necrosis, some cells showed loss of nucleus and nucleoli. Liver sinusoids were congested and infiltration by inflammatory cells. Silymarin Treated: Showed some cells with loss of nucleus but there were well defined cytoplasm. Occasional areas of kupffer cell proliferation were seen. ET1 Treated: Liver section showed normal lobular architecture with hardly any ascertainable regenerative activity. ET2 Treated: Liver section showed normal lobular architecture with some cells showing loss of nucleus, occasional areas of kupffer cell proliferation.
radicals, which forms covalent bond with membrane lipids and destroys the membrane integrity. The observation of increased MDA formation in hepatic cells after CCl₄ challenge is in accordance with the earlier reports which suggests involvement of trichloromethyl and trichloromethylperoxy radicals in the propagation of per-oxidation process [20]. The pretreatment with extract has prevented oxygen free radicals and thereby prevented the formation of peroxid radicals. This aspect of test extract also contributes to the hepatoprotectivity. The unpublished data on the hepatoprotective activity of this plant on other models like paracetamol and thiacetamide induced hepatotoxicity indicated that the hepatoprotectivity of the test extract is not model specific.

Thus, from the results of the present investigation, it may be concluded that the aqueous extract of the aerial parts of E. toxicaria possess significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity and antioxidant activity. The antioxidant potential may be attributed to the presence of polyphenolic compounds.

Further studies like isolation and characterization of the active principle(s) responsible for such activity are needed to confirm.

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