IJPT
1735-2657/08/71-25-30
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 7:25-30, 2008

RIDR

RESEARCH ARTICLE

Hepatoprotective and Antioxidant Activity of *Euphorbia tirucalli*

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Received March 26, 2007; Revised July 1, 2008; Accepted August 2, 2008

This paper is available online at *http://ijpt.iums.ac.ir*

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 sSerum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase ALP, biluribin, 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 inbuilt 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. Carbontetrachloride (CCl_4) is one such hazardous chemical which induces hepatopathy through membrane lipid peroxidation by its free radical derivative, ($CCl_3^{\cdot}$, $CCl_3O_2^{\cdot}$). 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 useful 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 itch and scorpion bites, it is also a warm rubefacient remedy in rheumatism and toothache [5]. Isolated compounds from the plant include cycloeuphornol, 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 decoction of the test plant is highly useful in treating jaundice. In addition the pharmacological profile of it is not completely established. Therefore this plant is taken for the present study. With this scientific information, the present study was designed with an aim to assess the antioxidant and hepatoprotective activity of the aqueous extract of aerial parts of *Euphorbia tirucalli* (ET), against CCl_4 induced liver damage.

MATERIALS AND METHODS

Plant material

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 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 (*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.

Animals

Adult Wistar rats (180-220 g) and Swiss albino mice (18-22g) 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. This was mixed with Phosphate buffer (2.5ml, 0.2M, pH6.6) and potassium ferricyanide (2.5ml, 1%). This mixture was incubated at 50°C for 20 minutes. A portion (2.5ml) of TCA (10%) was added to the mixture, which was then centrifuged at 3000rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled H_2O (2.5ml) and $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm.

Superoxide-anion scavenging activity

Measurement of superoxide anion scavenging activity was done based on the method described by Nishimiki [11] and slightly modified.

About 1ml of Nitroblue tetrazonium solution (156µM nitrobluetetarzolium in 100mM phosphate buffer, pH 7.4), 1ml nicotineamideADH solution (468µm in 100mM phosphate buffer, pH 7.4) and 0.1ml of sample solution of ET in water were mixed. The reaction is started by adding 100ml of phenazinemethosulphate (PMS) solution (60M) in 100mM phosphate buffer, (pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes and the absorbance at 560nm was measured against blank samples.

Hydroxyl radical scavenging activity

Hydroxyl radical (OH*) generation by Phenylhydrazine has been measured by the 2-deoxyribose degradation [12]. In 50mM phosphate buffer (pH 7.4) 1mM deoxyribose, 0.2mM phenylhydrazine hydrochloride and the extract at different doses (20-100µg) were added in a total volume of 1.6ml. Incubation was terminated after l hour or 4 hour and 1ml each of 2.8% trichloroacetic acid (TCA) and 1% (W/V) thiobarbituric acid (TBA) were added to the reaction mixture & heated for 10 minutes, in a boiling water bath. The tubes were then cooled briefly and absorbance taken at 532nm.

Acute Toxicity Studies

The test extract (Aqueous extract) was screened for acute toxicity on Swiss albino mice as per CPCSEA guideline No 420, i.e. fixed dose method (three animals in a group and repeated twice as per guideline 420). The animals were observed for 24 hours to assess immediate toxicity and for seven days for delayed mortality. Since, none of the animals died at 2500 mg/kg (oral) even after seven days, $1/20^{th}$ (125mg/kg) and $1/10^{th}$ (250mg/kg) of this cut off dose were selected for further studies.

Carbon tetra - chloride induced toxicity

The method of Ko et al [13] was used for screening the hepatoprotectivity of the test extract. Adult Wistar rats of either sex were randomly assigned into 5 groups of 6 animals. Group I and II serving as normal & intoxicated control and received only the vehicle (normal saline). Group III served as standard, was treated with Silymarin $(100 mg/kg BW p.o., for 3 days). The animals of Group IV and V received ET extract (125 mg/kg BW and 250mg/kg BW p.o., respectively) for 3 days. Twenty four hours after the last dosing, animals (except Group 1) were treated orally with CCl_4 (11 % *v/v* in olive oil) at a dose of 1 ml/kg BW. Whereas the animals of group I received 1 ml/kg of olive oil. Blood samples were collected after 24 hrs of CCl_4 intoxication by direct cardiac puncture under light ether anesthesia and animals were sacrificed by cervical decapitation and hepatic tissue was collected. Heparinized blood sample

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.

Treatment	Dose (µg)	Reducing Property (Abs)	Increase (%)	PMS-NaOH System (Abs)	Inhibition (%)
Control	-	0.229 ± 0.0012	-	0.863 ± 0.0076	-
SMS	25	0.417± 0.0105*	82	0.121± 0.0012*	86
ET	20	0.271±0.0241*	16	O.679±0.0043*	21
ET	40	0.284±0.0019*	20	0.612±0.0127*	29
ET	60	0.318±0.0312*	28	0.564±0.0987*	35
ET	80	0.349±0.0116*	52	0.491±0.0030*	43
ET	100	0.373±0.0053*	63	0 401±0.0310*	54

* → P – Value <0.001 Vs control group, Bonferrni test.
SMS → Sodium metabisulphate

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

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

*. P-Value <0.001 Vs 2-deoxyribose/phenylhydrazine treated, Bonferrni 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)

Groups	SGOT(U/L) Mean ± SE	SGPT(U/L) Mean ± SE	ALP (U/L) Mean ± SE	Cholesterol (mg/dl) Mean ± SE	Triglycerides (mg/dl) Mean ± SE	Total Bilirubin (mg/dl) Mean ± SE	Direct Bilirubin (mg/dl) Mean ± SE
GROUP 1	102 ± 5.48	51 ± 1.87	198±3.59	111±2.21	221±7.88	0.923±0.012	0.235±0.010
GROUP 2	355 ± 4.95	167 ±3.69	1036±6.32	179±2.27	766±4.57	5.036±0.035	1.84±0.056
GROUP 3	183 ± 4.93*	88 ±2.46*	483±2.52*	121±1.69*	463±2.03*	1.838±0.026*	0.248±0.006*
GROUP 4	284±2.822*	160±1.778*	951±4.906*	155±1.838*	699±3.149*	4.149±0.020*	0.713±0.005*
GROUP 5	213±2.671*	129±.693*	712±2.777*	142±2.362*	606±2.156*	3.002±0.030*	0.553±0.002*

Values are the Mean ± SEM of six rats/ treatment.
*. p<0.001 Vs CCl_4 treated group (group 2), Bonferrni test.
Group 1-Normal animals (Olive oil1ml/kg,p.o)
Group 2-CCl_4 (1ml/kg BW) treated animals
Group 3-CCl_4 + Silymarin (100mg/kg BW,p.o.) treated animals.
Group 4-CCl_4 + ET (125mg/kg BW, p.o.) treated animals.
Group 5-CCl_4 + ET (250mg/kg BW, p.o.) treated animals.

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 gluataic oxaloacetate transaminase (SGOT) and Serum glutamic pyruvic tranaminase (SGPT) [14], total bilirubin and direct billirubin [15], cholesterol, triglycerides and alkaline phosphate (ALP) contents.

Tissue glutathione measurements were performed using a modification of the Ellman procedure [16, 17]. Tissue samples were homogenized in ice cold TCA (lg tissue plus 10 ml 10 % TCA) in a homogenizer. Briefly after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2ml solution of DTNB (5,5 Dithio-bis 2- nitrobenzoic acid) (0.4 mg in 1 ml of

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).

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

*. P – Value <0.001 Vs normal saline – CCl_4 treated, Bonferrni test.
a. P – Value <0.01 Vs normal saline – CCl_4 treated, Bonferrni test.
Group 1-Normal animals (Olive oil1ml/kg,p.o)
Group 2-CCl_4 (1ml/kg BW) treated animals
Group 3-CCl_4 + Silymarin (100mg/kg BW,p.o.) treated animals.
Group 4-CCl_4 + ET (125mg/kg BW, p.o.) treated animals.
Group 5-CCl_4 + ET (250mg/kg BW, p.o.) treated animals.

1 % Sodium nitrate) was added and the absorbance at 412 nm was measured immediately after mixing.

Extent of lipid peroxidation was done by combining 1.0ml of biological sample (0.1 – 2.0 mg of membrane 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].

Statistical analysis

Data were analyzed by Bonferrni multiple comparison test using graph pad computer program. Results with $p<0.05$ were considered statistically significant.

RESULTS

In vitro Antioxidant Activity

Reducing power

Table 1 shows the dose dependant increase in the % of absorbance, indicating that the ET possesses dose dependant reducing power. All the doses of ET demonstrated greater absorbance than control & the difference were found to be significant ($p<0.001$), (Table 1).

Superoxide anion scavenging activity

In the PMS / NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS / NADH coupling reaction, reduces NBT. The decrease in absorbance at 560nm with EA thus indicates the consumption of superoxide anion radical in the reaction mixture, 100μg of the sample possess 54% of inhibition as compared to the standard Sodium metabisulphite (25μg) which showed 86% inhibition/scavenging activity (Table 1).

Hydroxyl radical scavenging activity

Results presented indicate that phenylhydrazine in solution generates OH* radicals as measured by 2-deoxy ribose degradation. It's found that 100μg of aqueous extract of ET and 25μg of sodium metabisulphate scavenge the OH* radicals and inhibit the production of TBA reactive material significantly over period 1 hour (Table 2).

Hepatoprotectivity

The estimated values of serum GOT, GPT, ALP, cholesterol, triglycerides, total Bilirubin and direct bilirubin values in control (saline + vehicle) group of rats are tabulated in table 3.

A remarkable elevation was observed in Serum GOT, GPT, ALP, cholesterol, triglycerides, total bilirubin and direct bilirubin values in CCI_4 intoxicated rats (Toxic Control group). In the groups treated with 125mg/kg and 250mg/kg of the extract, the above biochemical markers of hepatotoxicity were found to be decreased when compared to CCl_4 treated control group. Evidently, the hepatoprotective effects of higher dose of ET (250mg/kg) were near to that of standard i.e. Silymarin (100mg/kg). Both the doses of ET used in the study showed significant protective property than control. However the test extract was found to be less potent than that of standard drug.

The tissue glutathione was found to be depleted upon CCl_4 intoxication, indicate that the tissue damage is due to over powering the inbuilt free radical scavenger mechanisms. This tissue GSH depletion was inhibited by the pretreatment with test extract in a dose dependant manner. Similarly lipid peroxidation induced by CCl_4 treatment was reversed by test extract in a dose dependant manner. The results are compiled in table 4.

Histopathological reports show a promising response on treatment with aqueous extract of aerial parts of ET (Fig 1).

DISCUSSION

Since the extract has demonstrated dose dependant anti-oxidant activity in all the models of the study, the aqueous extract was taken for assessing the *invivo* hepatoprotective properties. Pretreatment with the test extract has reduced the elevated levels of biochemical markers of hepatoxicity. Further it was also observed that the tissue GSH depletion due to CCl_4 challenge was

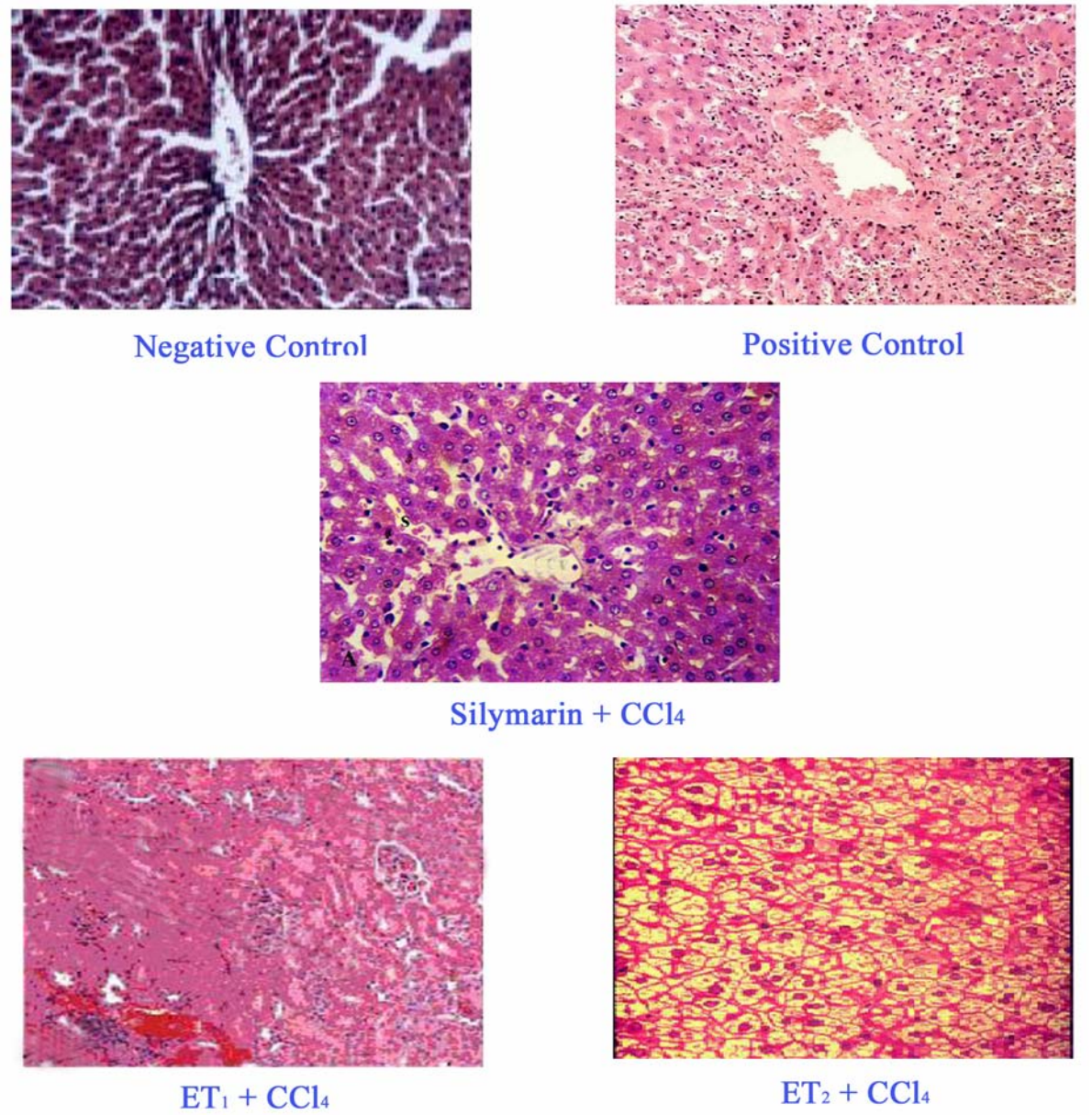

Fig 1. HISTOPATHOLOGY REPORT IN CCl_4 INDUCED HEPATOTOXICITY: Negative Control: Showed normal lobular architecture and normal hepatic cells with a well preserved cytoplasm and well-defined nuclei, nucleolus. Positive Control: Showed centrilobular necrosis, some cells showed loss of nucleus and nucleoli. 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.

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 *invitro* 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_2O_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 stand leakage [13]. ET extract reduced the super oxide 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 doest not interfere with the generation of the free radicals but it scavenges off the free radicals.

CCl_4 undergo hepatic metabolism to give rise to trichloromethyl radicals, which upon reacting with reactive oxygen species yields trichloromethyl peroxide

radicals, which forms covalent bond with membrane lipids and destroy the membrane integrity. The observation of increased MDA formation in hepatic cells after CCl_4 challenge is in accordance with the earlier report which suggests involvement of trichloromethyl and trichloromethylperoxy radicals in the propagation of peroxidation process [20]. 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. 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 ET 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 principal(s) responsible for such activity are needed to confirm.

ACKNOWLEDGEMENTS

The authors are very much thankful to Shri Sha. Bra. Chandramouleshwara Swamiji, President and Shri T.M. Chandrashekharaiah, Secretary, TMAE Society for their encouragement in carrying out this work.

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