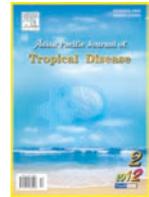




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Antioxidant and antihepatotoxic efficacy of methanolic extract of *Elephantopus scaber* Linn in Wistar rats

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ABSTRACT

Objective: To determine the *in vitro* and *in vivo* antioxidant and hepatoprotective activity of methanolic extract of *Elephantopus scaber* root against CCl₄ induced liver damage in rats. **Methods:** *In vitro* antioxidant activity was studied by determining superoxide scavenging, hydroxyl scavenging and Fe²⁺ ascorbate induced lipid peroxidation inhibiting activity of methanolic extract. The *in vivo* hepatoprotective activity was studied by estimating AST, ALT, ALP, GGT, total protein, albumin levels and by histopathological examination in CCl₄ toxicity induced experimental rats. The peroxidative hepatic damage was studied by assessing TBARS, CD, SOD, CAT and GSH in liver. **Results:** Methanolic extract of *Elephantopus scaber* root at doses of 75mg and 150mg/kg body weight significantly reduced the levels of AST, ALT, ALP & GGT and increased the level of TP and Albumin. The levels of TBARS and CD were decreased and the level of GSH increased. The levels of SOD and CAT were decreased. Histopathological changes induced by CCl₄ were reduced by the treatment of methanolic extract of *Elephantopus scaber* root. The effect was compared with reference drug curcumin. **Conclusions:** The antioxidant and antihepatotoxic activities of methanolic extract of *Elephantopus scaber* root was probably due to free radical scavenging activity.

1. Introduction

Carbontetrachloride is a toxic chemical biotransformed in liver to reactive metabolites such as trichloromethane (CCl₃) and trichloromethyl peroxy (CCl₃ O·O·) radicals which initiate peroxidation of membrane unsaturated fatty acids. This lipid peroxidation of membrane seriously impairs its function and produces liver injury[1]. CCl₄, the most widely used hepatotoxicant to date provides a relevant model for hepatotoxic studies[2]. Supplementation with exogenous antioxidants and phytochemicals from plant sources help to reduce the lipid peroxidation, oxidative damage and liver injury. The use of herbal drugs against hepatic disorders receives considerable attention these days. A number of medicinal plants and their polyherbal formulations are used in the treatment of hepatic disorders in the Indian system of

medicine[3–5].

Elephantopus scaber L. belonging to Asteraceae family is a wild shrub commonly found throughout the tropics is used as a diuretic, tonic, antihelminthic and in the treatment of hepatitis and liver complaints[6]. Teng–Khia–U is a Taiwan traditional medicine containing *Elephantopus scaber*, *Elephantopus mollis* and *Pseudoelephantopus spicatus* is used for treating nephritis, edema, dampness, chest pain, fever, cough of pneumonia and scabies. Researchers have showed that Teng–Khia–U possessed hepatoprotective and anti inflammatory activity[7,8]. The root powder suspension of *Elephantopus scaber* has been proved to possess hepatoprotective activity in CCl₄ induced liver injury[9]. The aim of the present study was to evaluate the antioxidant and antihepatotoxic activity of the methanolic extract of the root of *Elephantopus scaber*. Hepatoprotective effect of medicinal plants is exerted in part through their antioxidative and antilipid peroxidative properties[10,11]. The *in vitro* antioxidant activity was measured by its scavenging ability of hydroxyl radical, superoxide radical and inhibition of lipid peroxidation. The *in vivo* hepatoprotective activity

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and antioxidant activity was determined by administering methanolic extract of *Elephantopus scaber* L. root in CCl₄ induced rats. The liver function tests, antioxidant status in liver and histopathology of liver were done for assessment of hepatoprotective and antioxidant effect.

2. Materials and methods

2.1. Preparation of plant extract

Elephantopus scaber Linn was collected from Pathanamthitta district, Kerala, identified and a voucher specimen (SBS/BRL-1) was kept in School of Biosciences Mahatma Gandhi University, Kottayam. The roots of the plant were cut, washed and dried. The dried root was powdered extracted with methanol by soxhlet extraction for 24 hours. The extract was evaporated to dryness. The extract was presolubilised in 10% dimethyl sulphoxide for carrying out the *in vitro* antioxidant activities except for hydroxyl radical scavenging activity, where the extract was dissolved in warm phosphate buffered saline. For animal experiment the crude extract was prepared as a suspension in 5% Tween 80.

2.2. Animals

Male albino rats of Wistar strain weighing between 120 to 150g were used for the experimental purpose. The animals were housed in polypropylene cages and given standard pellet diet (M/S Hindusthan Lever Ltd. Bombay). Animal studies were conducted according to the Institute animal ethics committee regulations approved by the committee for the purpose of control and supervision of experiments on animals (CPCSEA) Reg No. B2442009/1.

2.3. In vitro assays

2.3.1. Assay of superoxide scavenging activity.

Superoxide radical (O₂⁻) generated from the photoreduction of riboflavin was detected by NBT reduction method^[12]. Curcumin was used as the standard.

2.3.2. Inhibition of lipid peroxidation.

Lipid peroxidation induced by the Fe²⁺ ascorbate system in rat liver homogenate was estimated by TBA reaction method^[12]. Curcumin was used as the standard.

2.3.3. Assay of hydroxyl radical scavenging activity.

Hydroxyl radicals generated from Fe³⁺-ascorbate-EDTA-H₂O₂ system (Fenton's reaction) was estimated by its degradation of the deoxyribose that resulted in thiobarbituric acid reactive substance (TBARS)^[12]. Curcumin was used as the standard.

2.4. In vivo assays

2.4.1. CCl₄ induced hepatotoxicity in rats

Experiments were conducted according to standard

protocol with minor modifications^[13]. Rats were divided into five groups with six animals in each group. Group I (Vehicle Control) animals served as control and received subcutaneous administration of liquid paraffin twice a week at the dose of 3ml/kg body weight of each animal for 14 d. Group II (Toxic Control) animals constituted the hepatotoxic group which received administration of LP+CCl₄ twice a week at the dose of 1 ml/kg body weight of CCl₄ in double the volume of LP at lower abdomen on every first and fourth days of the week. Group III, IV and V (Treated Groups) also received LP+CCl₄ as mentioned above. Group III received Curcumin at a dose of 75 mg/kg body weight orally. Group IV and V received methanolic extract of *Elephantopus scaber* at a dose of 75 mg and 150 mg/kg body weight orally. Animals were kept starved overnight on the 14th day. On the next day they were sacrificed by decapitation, by making an incision on jugular vein to collect blood. The liver tissue was dissected out, blotted off blood washed in saline and weighed instantaneously. This was kept in frozen containers and proceeded for biochemical estimations. Serum was separated and enzyme assay of AST, ALT, ALP, GGT, total protein and albumin were done. The liver was rapidly excised into small pieces, homogenized in buffer, centrifuged and supernatant used for hepatic antioxidant studies. Portions of liver were put in 10% formalin for histological examination.

2.4.2. Serum analysis

Serum AST, ALT, ALP, GGT, TP, Albumin were done using standard diagnostic kits.

The % protection was calculated using the formula

$$\% \text{ Protection} = \frac{\text{Toxic control} - \text{Treated Group}}{\text{Toxic control} - \text{Vehicle control}} \times 100$$

2.4.3. Tissue analysis for lipid peroxidation and antioxidant status

Liver tissues were homogenized with buffer in ice cold condition. The homogenates were centrifuged at 3000 r/min for 15 min and supernatants used for analysis. Lipid peroxidation was assessed in terms of thiobarbituric acid reacting substances and conjugated dienes. Changes in the antioxidant status were determined by estimating catalase, superoxide dismutase and reduced glutathione^[14].

2.4.4. Histopathology

Portions of liver were put in 10% formalin, embedded in paraffin and consecutive sections of 5 μm were cut mounted on microscopic slides and stained in haematoxylin and eosin for histopathological examination. The liver sections were graded numerically to assess the degree of histological features. Centrilobular necrosis or zonal necrosis, characterized by the damage of several liver cells around the central vein, fatty infiltration, prominent ballooning and bridging hepatic necrosis, a form of confluent necrosis of liver cells linking central vein to portal tracts or portal tracts to one another were prominent in histological studies^[15]. A combined score of centrilobular necrosis and

bridging hepatic necrosis was given a maximum value of 6. Descriptive modifiers such as mild, moderate, and severe was applied to activity and staging. The parameters were graded from score 0 to 6, with 0 indicating no abnormality, 1–2 indicating mild injury, 3–4 indicating moderate injury and 5–6 with severe liver injury.

2.5. Statistical analysis

The data obtained were analyzed for finding the variation between treated and control using one-way ANOVA followed by tukeys post hoc analysis. The level of significance was set as $P < 0.05$.

3. Results

3.1. In vitro antioxidant activity.

The methanolic extract of *Elephantopus scaber* root was found to be a scavenger of superoxide generated by photoreduction of riboflavin with an IC_{50} of $48 \pm 5 \mu\text{g/mL}$ while curcumin showed an IC_{50} of $9.8 \mu\text{g/mL}$. The methanolic extract of *Elephantopus scaber* root inhibited hydroxyl radicals generated by Fe^{3+} /ascorbate/EDTA/ H_2O_2 system with an IC_{50} of $72 \pm 12 \mu\text{g/mL}$ in comparison with curcumin with an IC_{50} of $38.2 \mu\text{g/mL}$. The generation of malondialdehyde (MDA) related substances that react with thiobarbituric acid (TBARS) were found to be inhibited by the extract. This significant lipid peroxidation inhibiting activity was found with the methanolic extract of *Elephantopus scaber* root with an IC_{50} of $103 \pm 18 \mu\text{g/mL}$ in comparison with curcumin with an IC_{50} of $16.5 \mu\text{g/mL}$ (Table 1).

3.2. Hepatoprotective activity.

The activities of ALP, AST, and ALT are shown in Figure 1; GGT, TP and albumin are shown in Table 2. The level of

these enzymes was significantly increased ($P \leq 0.05$) compared to vehicle control after the injection of CCl_4 . Treatment with curcumin at a dose of 75 mg/kg body weight, methanolic extract of *Elephantopus scaber* root at a dose of 75 mg/kg and 150 mg/kg body weight showed significant decrease ($P \leq 0.05$) compared to toxic control, with a protection of 81%, 59%, 66% in the level of ALT, 84%, 50%, 66% in the level of AST and 68%, 42%, 54% in the level of ALP.

Table 1.

In vitro antioxidant effect ($IC_{50} \mu\text{g/mL}$) of methanolic extract of *Elephantopus scaber* root.

Activities	Methanolic extract	Curcumin
Superoxide scavenging	48 ± 5	9.2 ± 1.1
Hydroxyl radical scavenging	72 ± 12	38.4 ± 6
Lipid peroxidation inhibiting	103 ± 18	16.5 ± 2.1

Values are mean \pm SD (n=3).

3.3. In vivo antioxidant activity.

There was a significant increase ($P \leq 0.05$) in the concentration of TBARS and CD during CCl_4 treatment as compared with the vehicle control. Administration of methanolic extract *Elephantopus scaber* root together with CCl_4 resulted in significant decrease ($P \leq 0.05$) of TBARS and CD in the liver compared with the corresponding CCl_4 intoxicated group. Curcumin also lowered the level of CD and TBARS when compared with the CCl_4 treated group. The activities of SOD and catalase in the liver tissues were significantly decreased in the CCl_4 treated rats as compared with the vehicle control. There was also a decrease in the content of GSH in the liver tissues of CCl_4 treated rats as compared with the vehicle control. Administration of methanolic extract of *Elephantopus scaber* root significantly ($P \leq 0.05$) restored the activities of the antioxidant enzymes and the level of glutathione to near normal compared with the corresponding CCl_4 intoxicated group. Curcumin also restored the activities of the antioxidant enzymes and the

Table 2.

Effect of methanolic extract of *Elephantopus scaber* root on liver function tests in carbon tetrachloride intoxicated rats.

Groups	GGT (U/L)	Total Protein (gm%)	Albumin (gm%)
Group 1 Control with normal saline	13.83 ± 4.91	6 ± 0.21	4.27 ± 0.22
Group 2 CCl_4 -treated	$64.83 \pm 7.98^*$	$5.25 \pm 0.12^*$	$3.21 \pm 0.12^*$
Group 3 CCl_4 +Curcumin	$30.66 \pm 3.38\#$	$5.76 \pm 0.31\#$	$3.96 \pm 0.12\#$
Group 4 CCl_4 + 75mg/kg of <i>E.scaber</i>	$42.00 \pm 6.25\#$	$5.25 \pm 0.15\#$	$3.52 \pm 0.12\#$
Group 5 CCl_4 +150mg/kg of <i>E.scaber</i>	$37.17 \pm 3.64\#$	$5.44 \pm 0.17\#$	$3.69 \pm 0.14\#$

Values are mean \pm SD, n=6. *: $P \leq 0.05$ vs normal control; #: $P \leq 0.05$ vs CCl_4 control.

Table 3.

Effect of methanolic extract of *Elephantopus scaber* root on antioxidant status of liver in carbon tetrachloride intoxicated rats.

Groups	SODU/mg protein	CATU/mg protein	GSHnmol/gtissue	TBARSMDAnM/mgprotein	CDmM/100g tissue
Group 1 Control with normal saline	5.24 ± 0.04	2.99 ± 0.02	5.05 ± 0.05	3.20 ± 0.02	63.85 ± 0.27
Group 2 CCl_4 -treated	$3.80 \pm 0.03^*$	$0.90 \pm 0.01^*$	$1.15 \pm 0.12^*$	$5.90 \pm 0.01^*$	$82.32 \pm 0.24^*$
Group 3 CCl_4 +Curcumin	$4.90 \pm 0.03\#$	$2.70 \pm 0.03\#$	$4.95 \pm 0.02\#$	$3.55 \pm 0.01\#$	$65.23 \pm 0.48\#$
Group 4 CCl_4 + 75mg/kg of <i>E.scaber</i>	$4.60 \pm 0.04\#$	$2.30 \pm 0.03\#$	$3.80 \pm 0.04\#$	$4.10 \pm 0.01\#$	$70.11 \pm 0.87\#$
Group 5 CCl_4 +150mg/kg of <i>E.scaber</i>	$4.80 \pm 0.05\#$	$2.50 \pm 0.03\#$	$4.86 \pm 0.03\#$	$3.90 \pm 0.01\#$	$68.01 \pm 0.93\#$

Values are mean \pm SD, n=6. *: $P \leq 0.05$ vs normal control; #: $P \leq 0.05$ vs CCl_4 control.

level of glutathione compared with the corresponding CCl₄ intoxicated group (Table 3).

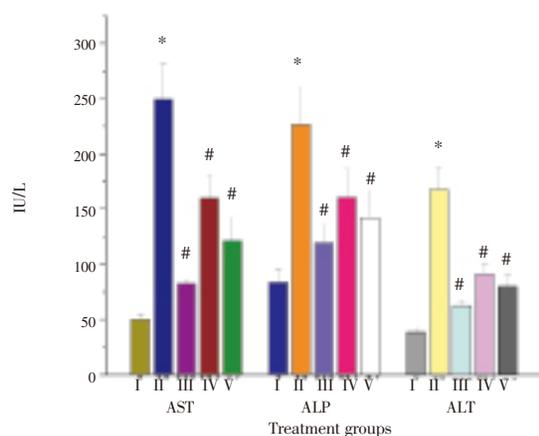


Figure 1. Estimation of biochemical parameters AST, ALP and ALT. I: Normal control; II: CCl₄ treated group; III: CCl₄+curcumin; IV: CCl₄+methanolic extract 75mg/kg; V: CCl₄+methanolic extract 150mg/kg. Values are mean±SD, n=6, *: $P \leq 0.05$ vs normal control, #: $P \leq 0.05$ vs CCl₄ control.

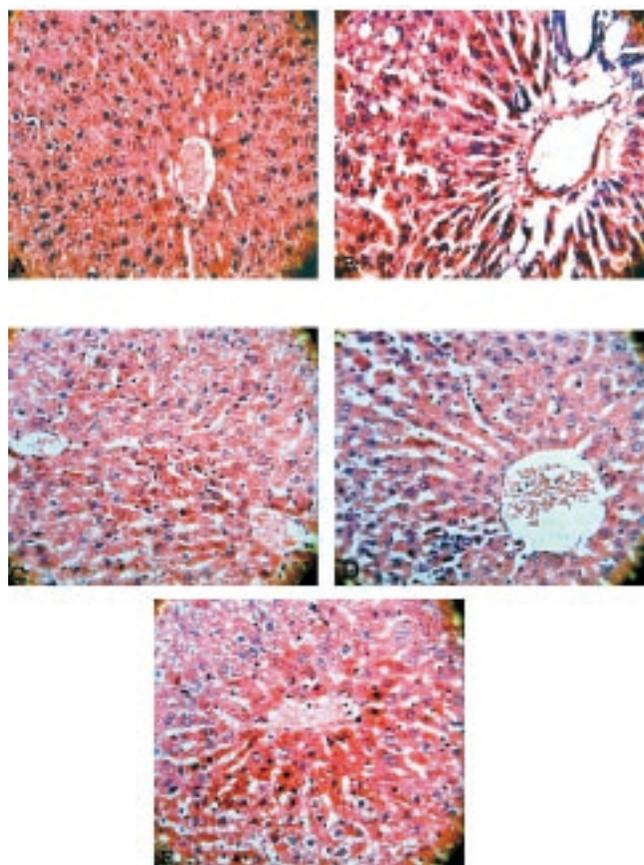


Figure 2. Histopathological changes occurred in rats during CCl₄ intoxication and prevention by the treatment with methanolic extract of *Elephantopus scaber* root (hematoxylin and eosin, original magnification 400x).

A: normal control; B: CCl₄ control; C: CCl₄+methanolic extract 75mg/kg; D: CCl₄+methanolic extract 150mg/kg; E: CCl₄+curcumin 75mg/kg.

3.4. Histopathological analysis

Histopathological study of liver from group I animals

showed a normal hepatic architecture (Figure 2A). In CCl₄ treated group, significant ($P \leq 0.05$) hepatic toxicity was evidenced by bridging hepatic and centrilobular necrosis, ballooning and fatty infiltration of the liver scoring 5.6 ± 0.4 (mean±SD, n=3) (Figure 2B). In curcumin treated and *Elephantopus scaber* root treated rats, at doses 75 mg/kg and 150 mg/kg body weight liver exhibited significant ($P \leq 0.05$) protection from CCl₄ damage showing an almost normal architecture barring a little deformity of hepatocytes with pyknosis and clearing of cytoplasm with scores 1.8 ± 0.7 ; 2.5 ± 0.5 ; 2.1 ± 0.7 (mean±SD, n=3; $P \leq 0.05$) respectively (Figure 2C–2E).

4. Discussion

The pathological processes in liver injury and its complications are closely related with elevated ROS formation. Oxidative stress is caused by a disturbance of the balance between the anti oxidant defense mechanism of the human organism and the level of ROS and has been associated with many pathological disorders such as atherosclerosis, diabetes, liver disease and cancer^[16].

Any compound with antioxidant property can have protective effect against damage caused by free radicals and reactive oxygen species. Studies indicate that reactive oxygen species such as superoxides, hydrogen peroxides and hydroxy radicals play a central role in the mechanism of DNA damage and cytotoxicity^[16,17]. The superoxide generation by the reaction of photoreduced riboflavin and oxygen is inhibited by the methanolic extract of *Elephantopus scaber* root. The generation of malondialdehyde and related substances from lipid extract react with thiobarbituric acid was found to be inhibited by the methanolic extract of *Elephantopus scaber* root. The degradation of deoxyribose to TBARS by the hydroxyl radicals generated from Fe³⁺-ascorbate-EDTA-H₂O₂ system was markedly decreased by the methanolic extract of *Elephantopus scaber* root. Hence the methanolic extract of *Elephantopus scaber* root has proved to be an efficient antioxidant in the *in vitro* model.

In the *in vivo* experimental model of rats, CCl₄ intoxication generates reactive metabolites such as trichloromethyl (CCl₃) and trichloromethyl peroxy (CCl₃O-O·) free radicals which produce peroxidation of membrane unsaturated fatty acids leading to liver injury. CCl₄ is a potent hepatotoxin and a single exposure can rapidly lead to severe centrilobular necrosis and steatosis¹. The liver enzymes being cytoplasmic in origin and liver injury cause the entry of these enzymes to the circulatory system. The elevation of these liver marker enzymes and the decrease of protein and albumin caused by the CCl₄ induced liver damage is significantly decreased by the administration of the methanolic extract of *Elephantopus scaber* root. Curcumin also showed better protection towards CCl₄ induced liver damage.

The antioxidant enzymes SOD, catalase and peroxidases

constitute a mutually supportive team of defense against reactive oxygen species. The decrease in the activity of SOD in the liver of CCl₄ treated rats may be due to the increased lipid peroxidation or crosslinking with malondialdehyde. This will cause an increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. Depletion of GSH results in enhanced lipid peroxidation, which in turn caused increased GSH consumption. The extract prevented the rise of TBARS and CD and decrease of GSH. The decrease in the level of antioxidant enzymes like SOD, and catalase is prevented by the administration of methanolic extract. The histological changes induced by the CCl₄ treatment, indicated by the hepatic necrosis and its conversion to normalcy by the administration of methanolic extract of *Elephantopus scaber* root.

In vitro antioxidant activity of methanolic extract of *Elephantopus scaber* root and the result of serum biochemical parameters, level of antioxidant enzymes, level of TBARS and CD, level of GSH and histopathological studies in the *in vivo* model strongly support the highly potent antioxidant and hepatoprotective activity of methanolic extract of *Elephantopus scaber* root. The study supports to the use of *Elephantopus scaber* in the treatment of hepatitis in Taiwanese folk medicine, Teng-Khia-U. The methanolic extracts were known to possess sesquiterpene lactones^[18] which might be responsible for the hepatoprotective and antioxidant activity. Further studies on the active compound responsible for this activity are in progress. In short the methanolic extract of *Elephantopus scaber* root has significant hepatoprotective activity in rats. This effect is probably mediated through its significant antioxidant activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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