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Antihepatotoxic effect of *Elephantopus scaber L.* on carbon tetrachloride-induced hepatotoxicity in rats

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Abstract
Elephantopus scaber was used in folk medicine in several countries to treat different diseases. Liver diseases are major World wise health Problems. The Present aim of the study was evaluate The Antihepatotoxic effects of different fractions of *Elephantopus scaber* against Carbon tetrachloride (CCl_4) induced hepatic damage in Rats. All the fractions were given orally in different doses (125mg/kg, 250mg/kg, 500mg/kg). The Antihepatotoxic effect was assessed by measuring serum parameters like aspertate transaminase (AST), alanine transaminase (ALT), Alkaline Phospatase (ALP) and total bilurubin. All the fractions of *Elephantopus scaber* showed Antihepatotoxic effect.The ethanol fraction was shows significant percentage protection than compared to other fractions. Therefore, our study supports the isolation and use of active constituents from Ethanol fraction of *Elephantopus scaber* in treating of liver disease.

Keywords: *Elephantopus scaber*, Antihepatotoxic effect , Carbon tetrachloride (CCl_4)

INTRODUCTION

Liver damage is one of the most serious diseases which has accompanied the adoption of modern food styles as well as exposure to many environmental pollutants and intensive intake of medications. Various xenobiotics are known to cause hepatotoxicity, one among them is carbon tetrachloride (CCl4) that may cause lipid peroxidation [1, 2]. Oriental herbal medicines have recently attracted the interest of modern scientific communities as alternative therapy. There has been a sharp upward trend in the use of phytomedicines over the last decades in Europe and USA [3,4].the root decoction of *Elephantopus scaber* is widely used to treat diarrhea,dysentery,stomach troubles and blood vomiting in tuberculosis in Nepal, [5,6].Hot water extract of root has been used to remedy fever [7].Root paste has been used externally as anti venom, antiseptic for cuts and wounds, lesions for chicken pox, antipyresis (2 teaspoonfuls of the root paste three times a day for 2-3 day) while its fresh roots are chewed to treat cough, cold and headache The current study was designed to investigate the potential antihepatotoxic effect of *Elephantopus scaber* in CCl_4-induced hepatotoxicity and liver damage in rats.

MATERIALS AND METHODS

Drug and Chemicals

Silymarin, Carbon tetrachloride (CCl_4) was purchased from Sigma chemicals, USA., Aspartate Transaminase (AST), Alanine Transaminase (ALT), Serum Alkaline Phosphatase (ALP), Serum Total bilirubin (T.Bil) kits were purchased from Span diagnostics Ltd, Gujarat, India. All other chemicals used were of analytical grade.

Plant Material and Preparation of extracts

The *Elephantopus scaber* was collected from Puchikapadu, West Godavari District, Andhra Pradesh, India, during the month of April 2010. The authentication of the above plant was done by Dr. P. Prayaga Murthy, Department of Botany, Andhra University, Visakhapatnam. Freshly collected *Elephantopus scaber* plant was dried under shade. The coarse powdered was macerated in 70% v/v ethanol. The liquid extract was collected and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and it was re dissolved in water then fractionated by using hexane and ethyl acetate. The liquid (hexane soluble, ethyl acetate soluble) was collected separately and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and the coarse powder after filtration from 70% v/v ethanol was dried and again macerated in methanol. The liquid extract (methanol soluble) was collected and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and then the different extracts were for further investigation.

Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250 g were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2°C having 50±5% relative humidity with 12-h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and

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Acute toxicity studies

Acute toxicity study was conducted according OECD Guide lines No.423. After fasting overnight, mice were administered with extracts of *Elephantopus scaber* in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed continuously for 1 h and then hourly for 6 h and finally after every 24 h up to 15 days for any toxicological symptoms or mortality

Assessment of antihepatotoxic effect against CCL_4 liver intoxication

Carbon tetrachloride intoxication in rats is an experimental model widely used to study necrosis and steatosis of liver [8,9]. (Rubin et al., 1963; Recnagal, 1983).The animals were divided in to 7 groups, each consisting of 6 animals. The standard and test group animals were treated respectively with 50 mg/kg dose of Silymarin and 500 mg/kg dose of 70% ethanol extract and 100 mg/kg dose of methanol, ethyl acetate, hexane extracts of *Elephantopus scaber* for 5 days. On 6th day, 1hr after treatment with standard and test doses, the animals were intoxicated with CCl_4: liquid paraffin (1:1) (1ml/kg,p.o). Control group also received 1ml/kg dose of CCl_4 on 6th day. On 7th day the blood samples were collected and analyzed for biochemical parameters like serum enzymes, Aspartate transaminase (AST), Alanine transaminase (ALT) were estimated by Reitman and Frankel, 1957 method, Serum Alkaline Phosphatase (ALP) by King and Armstrong, 1980 method and Serum Total bilirubin (T.Bil) by Jendrassik and Grof, 1938 method by using commercial reagent kits in Autoanalyzer (RM4000, Biochemical systems International, Italy) [10,11,12]. (Reitman, S and Frankel, S 1957; King,E.J and Armstrong,A.R 1934; Jendrassik, L and Gróf, P 1938).

Calculation of 50% Inhibition Concentration

The optical density obtained with each concentration of the extract/ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

Statistical Analysis

Values were expressed as means ± standard deviation. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA and linear regression analysis was used to calculate IC_{50} values.

RESULTS AND DISCUSSION

Antihepatotoxic effect against CCl_4 intoxication

Results in table (1) showed that s.c. injection of CCl4 induced a significant increase in serum level of liver enzymes at the end of the experiment .Administration of silymarin on 6 day induced significant reduction in the blood levels of AST, ALT and ALP, but did not significantly affect the ALP levels. *Elephantopus scaber* fractions significantly reduced the blood levels of the AST, ALT ALP and Total biliruibin compared to the values of CCl4-treated group .the ethanol fraction of *Elephantopus scaber* was shows significant activity than other fractions the results ware tabulated below.

Table 1. Percentage protection produced by extracts of *Elephantopus scaber* on serum parameters against CCl_4 intoxication in rats.

Name of the group and extract	serum parameters			
	AST (U/L)	ALT (U/L)	ALP(U/L)	Total bilirubin (mg/dl)
Group – A 5% gum acacia (Vehicle)	96.17 ±2.85	56.00 ±1.46	217.50 ±1.06	0.17 ± 0.01
Group - B CCl_4 (1ml/kg,p.o)	194.33± 2.73	141.00± 1.88	471.50 ± 12.16	0.27 ± 0.0
Group-C/ Silymarin (50mg/kg)	102.50± 1.61 (83.74)	60.83 ±1.08 (93.76)	245.33 ±2.70 (89.10)	0.17 ± 0.01 (95.0)

Table 2. The effect of *Elephantopus scaber* hexane fraction on CCl4-treated induced alterations in serum hepatic enzymes including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilurubin levels

Concentrations		Serum parameters			
		AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)
(125mg/kg)	Mean±S.E.M	178.40±0.50	127.93±0.27	349.90±0.75	0.23±0.01
	Percentage protection	16.22	15.37	47.87	40.00
(250mg/kg)	Mean±S.E.M	168.10±0.24	115.15±1.47	333.27±0.48	0.22±0.00
	Percentage protection	26.72	30.41	54.96	50.00
(500mg/kg)	Mean±S.E.M	143.22±0.32	102.56±0.91	316.23±1.19	0.21±0.02
	Percentage protection	52.06	47.87	61.12	60.00

Table 3. The effect of *Elephantopus scaber* Ethyl acetate fraction on CCl4-treated induced alterations in serum hepatic enzymes including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilurubin levels

Concentrations	Serum parameters				
		AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)
(125mg/kg)	Mean±S.E.M	177.95±0.30	127.35±0.24	348.9±0.29	0.23±0.00
	Percentage protection	16.68	16.05	48.26	40.00
(250mg/kg)	Mean±S.E.M	167.97±0.34	114.43±1.43	332.95±0.64	0.22±0.00
	Percentage protection	26.85	31.27	54.54	50.00
(500mg/kg)	Mean±S.E.M	142.04±0.45	101.89±0.86	315.69±0.96	0.21±0.00.0
	Percentage protection	53.27	46.01	61.34	60.00

Table 4. The effect of *Elephantopus scaber* Ethanol fraction on CCl4-treated induced alterations in serum hepatic enzymes including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilurubin levels

Concentrations	Serum parameters				
		AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)
(125mg/kg)	Mean±S.E.M	169.27±0.54	121.14±0.39	320.92±0.37	0.21±0.00
	Percentage protection	25.52	23.36	59.28	60.00
(250mg/kg)	Mean±S.E.M	154.76±0.45	107.88±0.35	315.02±0.68	0.20±0.00
	Percentage protection	40.24	38.96	61.60	70.00
(500mg/kg)	Mean±S.E.M	124.95±0.17	90.07±0.55	298.96±0.46	0.19±0.00
	Percentage protection	70.68	59.91	67.92	80.00

Table 5. The effect of *Elephantopus scaber* Methanol fraction on CCl4-treated induced alterations in serum hepatic enzymes including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilurubin levels

Concentrations	Serum parameters				
		AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)
(125mg/kg)	Mean±S.E.M	169.56±0.63	121.89±0.63	322.36±0.33	0.21±0.00
	Percentage protection	25.23	22.48	58.71	60.00
(250mg/kg)	Mean±S.E.M	156.67±0.27	109.67±0.16	319.90±0.60	0.20±0.00
	Percentage protection	38.36	36.85	59.68	70.00
(500mg/kg)	Mean±S.E.M	128.74±0.43	92.97±0.37	301.81±0.29	0.19±0.00
	Percentage protection	66.81	56.50	66.80	80.00

Note:All groups were compared with CCl_4 group. Values are mean ± S.E.M., *n* = 6 animals per group.Values in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatoprotection. The percentage of the protection is calculated as 100 × *(*values of CCl_4 -values of sample)/ (values of CCl_4 control - values of vehicle).

The acute toxicity studies revealed that the plant extracts at an oral dose of 2000 mg/kg produced no toxicity. The results clearly indicated the non toxicity of plant extract. The Preliminary Phytochemical studies showed that the plant extracts possess Alkaloids, Terpenoids and fixed oils.

Liver damage is always associated with cellular necrosis, increase in tissue LP and depletion of reduced liver glutathione. In addition, elevated levels of hepatic serum enzymes are indicative of cellular leakage [13]. Among xenobiotics, CCl4 represents the main cause of acute liver injury through its bioactivation to trichloromethyl free radicals that cause LP and produces hepatocellular damage [14,15]. In our study, CCl4 induced severe liver damage as evidenced by the signifi cant elevation of serum levels of ALT, AST, ALP and LDH that indicates the severity of liver injury [16].The plant extracts showed hepatoprotective activity against CCl_4 intoxication. When compared to silymarin the plant extracts showed antihepatotoxic effect. Based on AST,ALT,ALP, Total bilirubin levels the ethanol fraction showed better activity compared with other fractions. Studies are in progress to establish the mechanism of action and isolation of bioactive molecule

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