

TO INVESTIGATE LEUCAS CEPHALOTES FOR ITS ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY AGAINST CCL₄ INDUCED HEPATOTOXICITY IN SWISS ALBINO MICE

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ABSTRACT

Leucas cephalotes is a weed flora of wet season and has been traditionally used as mild stimulant, diaphoretic and used in fever and liver disorder. The object of the present study is to investigate the methanolic extract of the plant *Leacus Cephalotes* for hepatoprotective activity against carbon tetra chloride-induced liver damage in swiss albino mice and antioxidant activity by hydrogen peroxide scavenging assay in dose dependant manner. Methanolic extract of plant *Leacus cephalotes* investigate for hepatoprotective activity against carbon tetrachloride induced hepatotoxicity using biochemical markers Serum glutamate pyruvate transaminase- SGPT, Serum glutamate oxaloacetate transaminase- SGOT, serum bilirubin, Serum alkaline phosphatase- SALP. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Antioxidant activity investigated by invitro hydrogen peroxide scavenging assay. Biochemical studies of blood samples of methanolic extract of *Leacus Cephalotes* showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells, the extract of above plant could afford significant protection against CCl₄ induced hepatocellular injury. The methanolic extract from *Leucas cephalotes* exerts significant antioxidant effects, which were comparable with standard drugs. Administration of methanolic extract of the plant *Leacus Cephalotes* protect the liver from toxic effects of carbon tetra chloride by reducing the elevated levels of Serum enzymes which shows the significant protection against CCl₄ induced hepatocellular injury.

KEYWORDS

Leucas cephalotes, Hepatoprotective activity, Antioxidant activity.

INTRODUCTION

Plants are one of the most important sources of medicines. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice.¹ Free radicals, from both endogenous and exogenous sources, are implicated in the etiology of several degenerative diseases, such as coronary artery diseases, stroke, rheumatoid arthritis, diabetes and cancer. High consumption of fruits and vegetables is associated with low risk for these diseases, which is attributed to the antioxidant vitamins and other phytochemicals. There is a great deal of interest in edible plants that contain antioxidants and health – promoting phytochemicals, in view of their health implications.²

Leucas Cephalotes (Labiatae or Lamiaceae) weed flora of wet season mainly found in North India. Common name of it are 'Kubo or Kubi' in traditional medicine of Gujarat. The synonym of plant in Sanskrit is "Dronapushpi. The genus *Leucas* includes about 100 Asiatic and African species. It is a valuable drug for snake bite. The plant is useful in bronchitis, inflammation, asthma, dyspepsia, paralysis and leucoma. The leaves are useful in fever and urinary discharge.³

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the

biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. Liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is not much drug available for the treatment of liver disorders. Therefore many folk remedies from plant origin are tested for its potential hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl₄) induced hepatotoxicity model is widely used for the study of hepatoprotective effect of drugs and plants.⁴

EXPERIMENTAL

Collection and identification of the plant material

The whole plant of *Leucas Cephalotes* was collected from the Dist. Ambikapur (C.G.) in the month of December 2010 and authenticated at Safia college of Science Bhopal, Madhya Pradesh. The voucher specimen (**235/BOT/SAFIA/11**) was deposited in Department of Pharmacognosy, T I T College of Pharmacy, Bhopal, Madhya Pradesh.

Preparation of Leucas Cephalotes powder

The whole plants of *Leucas Cephalotes* were dried in shade and then powder with a mechanical grinder. The powder was passed through sieve no. 40 and stored in a labeled air tight container for further studies.

Extraction of plant material

Maceration process involves separation of medicinally active portions of the crude drugs. It is based on the immersion of the crude drugs in the bulk of solvent or menstrum. Solid drug material was taken in a Stoppard container with about 750ml of the menstrum and allowed to stand for at least three to seven days in a warm place with frequent shaking. The mixture of crude drug containing solvent was filtered until most of the liquid drains off. The filtered and the washing were the combined to produce 1000ml of the solution. This solution was kept aside for solidification and after it was dry on hot air oven for 30 minutes at 40°C. The extract collected in a dry dark color bottle.⁵

Hydrogen peroxide scavenging assay

The ability of extracts to scavenge hydrogen peroxide was determined by little modification here the solution of hydrogen peroxide (100mM) was prepared instead of 40mM in phosphate buffer saline of (PH 7.4), at various concentration of aqueous and ethanolic extract (100 -1000 µg/ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. In case of control takes absorbance of hydrogen peroxide at 230 nm without sample extracts. Percent inhibition of Hydrogen Peroxide scavenging activity was calculated using the following formula:-

$$\% \text{ scavenged } [H_2O_2] = (A_0 - A_1) / A_0 \times 100$$

Where A₀ is the absorbance of the control and A₁ is the absorbance of extract/standard taken as Gallic acid (10 - 100 µg/ml).^{6, 7.}

Hepatoprotective activity

Animal handling and care

Healthy 6-8 week old male mice (30-35g) were obtained from the animal house of Pinnacle Biomedical Research Institute

(PBRI) Bhopal (M.P.) India. Animals were housed in a group of four in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were further divided in nine groups with six animals in each group. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1283/c/09/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/140.

Experimental protocol

Mice were divided into six groups of six animals in each group. Group 1 served as normal control and was given distilled water orally. Group 2, the drug control group was given the standard drug silymerin orally for 7 days and sacrificed on the following day. Group 3 served as negative control group and was given CCl₄ orally for 7 days. In group 4, 5 and 6 different doses of plant extract respectively 100, 200 and 400 mg/kg was administered for seven consecutive days followed by a single oral dose of CCl₄ on the 7th day. Animal were sacrificed by anesthesia 16th hr after CCl₄ administration, the liver perfused with saline was dissected out and processed immediately for biochemical assay and a small part of liver stored in 10% formaldehyde for histological examination.^{8, 9.}

Biochemical determination

The biochemical parameters (serum enzymes) SGOT, SGPT, ALP and total bilirubin were assayed spectrophotometrically using a commercially available assay kits according to the manufacturer's protocol.⁸

Statistical analysis

IC₅₀ values, from the in vitro data, were calculated by regression analysis. Statistical comparisons of in vivo data were made by means of Student's t-test; *P*<0.05 was regarded as significant.⁸

RESULTS AND DISCUSSION

1. Hydrogen peroxide scavenging assay

The plant of *Leucas cephalotes* is an indigenous herb which was chosen for this study. The plant belongs to the family Lamiaceae. The scanty availability of information on this plant facilitates the study on it since ages various part of this part is being used for their medicinal use. Literature

review revealed that various chemical and biological investigations were carried out with this plant. Hydrogen peroxide scavenging assay was performed in methanolic extract of *Leucas cephalotes*. Ascorbic acid was taken as standard. Standard curve of ascorbic acid was shown in table no. 1 and figure no. 1. The observation of extract was shown in table no. 2 and 3 and figure no. 2.

Table 1
Concentration and absorbance of standard ascorbic acid for Hydrogen per oxide scavenging activity

S.no.	conc. (µg/ml)	% inhibition of hydrogen peroxide
1	2	1.61
2	4	3.82
3	6	5.43
4	8	6.62
5	10	7.32

Figure 1
Standard curve of Ascorbic acid

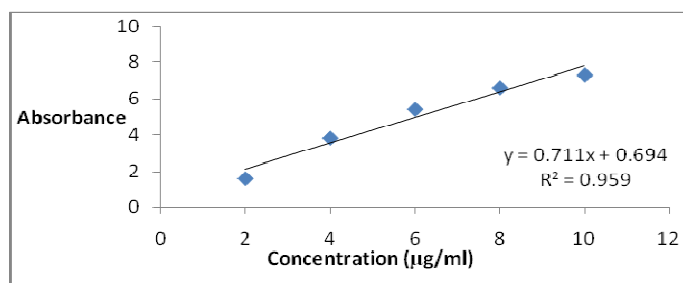


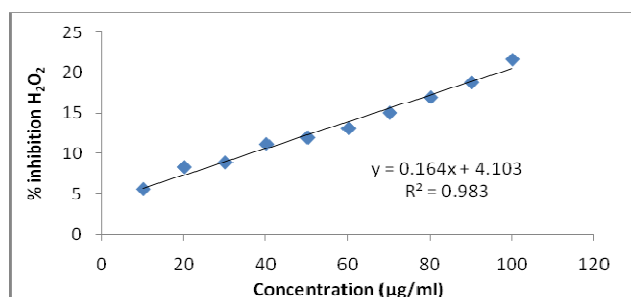
Table 2
Observation of extract for Hydrogen per oxide scavenging activity

S.no.	concentration	with H2O2	without H2O2	control	with – with out	% inhibition
1	10	0.834	0.004	0.879	0.83	5.574516
2	20	0.812	0.006	0.879	0.806	8.304892
3	30	0.809	0.008	0.879	0.801	8.87372
4	40	0.793	0.012	0.879	0.781	11.14903
5	50	0.789	0.015	0.879	0.774	11.94539
6	60	0.781	0.017	0.879	0.764	13.08305
7	70	0.765	0.018	0.879	0.747	15.01706
8	80	0.751	0.021	0.879	0.73	16.95108
9	90	0.743	0.029	0.879	0.714	18.77133
10	100	0.721	0.032	0.879	0.689	21.61547

Table 3
IC₅₀ of Ascorbic acid and methanolic extract

S.no.	Sample	IC 50
1	Ascorbic acid	69.34 µg/ml
2	Extract-2	279.689 µg/ml

Figure 2
Curve of extract for H₂O₂ scavenging activity



2. Hepatoprotective activity

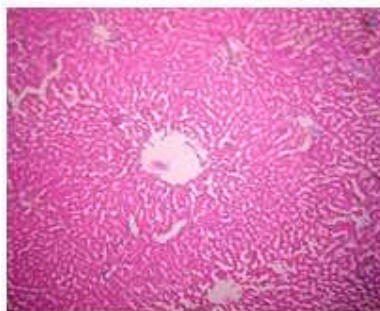
The methanolic extract of plant *Leacus cephalotes* showed a good hepatoprotective activity at a dose of 400mg/kg b.w. Comparative histopathological study of the liver from different group of mice corroborated the hepatoprotective efficacy of *Leacus cephalotes*. Because of hepatotoxic effect of CCl₄ is due to oxidative damage of free radical generation, antioxidant property is claimed to be one of the mechanism of hepatoprotective drugs. Further flavonoids have been suggested to act as antioxidants by free radical scavenging. Thus the hepatoprotective activity of *Leacus cephalotes* may be attributed to the

presence of flavonoids, through it is to be confirmed. The present study had been attempted to demonstrate the role of hepatoprotective activity of methanolic extract of plant *Leacus cephalotes* belonging to family Lamiaceae in CCl₄ induced hepatotoxicity at different doses. This plant shows better hepatoprotective activity at dose 400mg/kg b.w. The observation of activity was shown in table no. 4. The results of histopathological examinations have demonstrated in figure no.

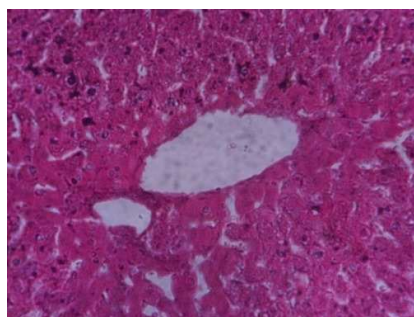
Table 4
Effect of Methanolic extract of plant *Leacus cephalotes* on various biochemical parameters in mice with CCl₄ induced hepatotoxicity

Groups	SGPT(IU/L)	SGOT(IU/L)	ALP(Ka)	Bilurubin(mg/dL)
VEHICLE	36.51±3.61	44.11±3.68	10.13±1.69	0.435±0.053
STANDARD(SILYMERIN)	48.42±2.53	52.36±4.55	12.5±1.46	0.492±0.070
CONTROL	163.06±11.18	162.35±11.46	49.11±2.40	5.83±0.640
Extract 100mg/kg	133.38±6.26	131.48±2.92	41.05±2.54	3.31±0.446
Extract 200mg/kg	90.06±8.19	88.06±3.60	20.06±1.68	1.90±0.186
Extract 400mg/kg	61.73±4.88	70.91±5.12	16.35±0.98	0.609±0.068

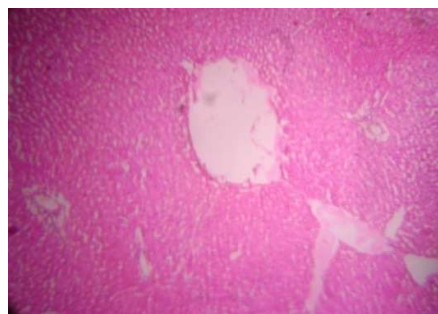
Figure 3
Histology of Liver



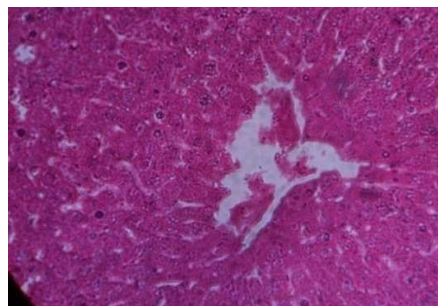
Micro view of liver tissue of normal mice



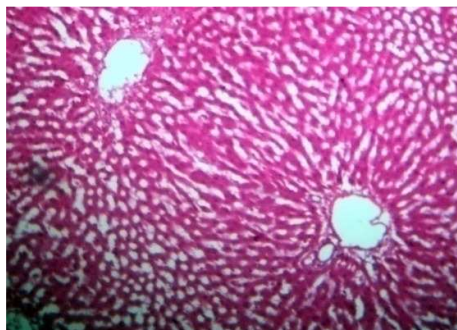
Micro view of liver tissue of Silymerin treated mice



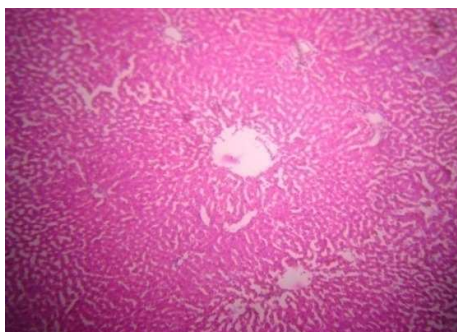
Micro view of liver tissue of CCl₄ treated mice



Micro view of liver tissue of Extract 100mg/kg treated mice



Micro view of liver tissue of Extract 200mg/kg treated mice



Micro view of liver tissue of Extract 400mg/kg treated mice

CONCLUSION

The plant of *Leucas cephalotes* exhibits a set of diagnostic characters which will help to identify the drug in dried condition. It has been concluded from this study that estimation is highly essential for raw drugs or plant parts used for the preparation of compound formulation drug. The periodic assessment is essential for quality assurance and safer use of herbal drugs.

Plants produce a variety of antioxidants against molecular damage from reactive

oxygen species [ROS], produced by macrophages. Phenolic compounds are the major class of plant-derived antioxidants. Among the various phenolic compounds, flavonoids are perhaps the most important group. In the present study, the hepatoprotective activity and antioxidant activity of *Leucas cephalotes* might be due to the presence of flavonoids in the methanolic extract.

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