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Der Pharma Chemica, 2010, 2(5): 458-462
(<http://derpharmachemica.com/archive.html>)



ISSN 0975-413X
CODEN (USA): PCHHAX

Hepatoprotection by *Leucas Aspera*

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ABSTRACT

The aim of the present work is to study the effect of *Leucas aspera* on CCl_4 induced liver damage. The acute toxicity study of this plant material indicates that the plant material has no any toxic effect. Hepatotoxicity is produced by single intraperitoneal dose of CCl_4 which alters the level of enzyme markers. The blood and tissue biochemical parameters were reported during animal trials. From these observations it is clear that this *Leucas aspera* has ability to regenerate liver cells. The *Leucas aspera* plant material treatment in the form of aqueous slurry shows good matching of these parameters with normal control group. This plant material impairs normal liver functioning including distinct toxic changes in hepatocytes. Thus this plant may be used as good hepatoprotectant..

Keywords: hepatoprotectant, regeneration of liver cells, *Leucas aspera*.

INTRODUCTION

Leucas aspera is a commonly occurring plant that grows as a weed on wastelands and roadsides all over India. The plant is used as an insecticide and indicated in traditional medicine for coughs, colds, painful swellings, and chronic skin eruptions.[1] The compounds isolated from the plant includes long-chain aliphatic compounds, a triterpene-leucolactone, sterols- sitosterol, campesterol, stigmasterol and a novel phenolic compound.[2-5]. The present study is aimed to examine the hepatoprotective activity of *Leucas aspera* in carbon tetrachloride induced hepatotoxicity in rats.

MATERIALS AND METHODS

Leucas aspera plant material was collected from Avasari Forest Park, Pune, washed thoroughly and dried at room temperature in shade. They were powdered, sieved through sieve of mesh to 85 (BSS) and stored in airtight containers. Swiss albino mice were used for toxicity study, while the hepatoprotective study was carried out in adult male and female Wistar rats (130-150 g)

procured from Raj Biotech (INDIA) Pvt. Ltd, Pune 411 038. The rats were housed in clean polypropylene cages and fed with commercial AMRUIT rat feed and water *ad libitum*.

Acute toxicity study was carried out for doses 2, 4 and 6 g/kg whole plant powder with distilled water as a vehicle. The animals were continuously observed for 1 h, then frequently for 24 h, and thereafter once per day for successive 14 days. There was no abnormality observed in any of the three groups.[9-12]

One-tenth of the maximum tested dose (i.e. 0.4 g/kg) of the plant material was selected for the evaluation of antihepatotoxic activity. For this study, a total of 60 rats were divided into five groups (n=12 in each group). Group I (vehicle control), Group II (CCl₄ control), Group III (CCl₄ Natural Recovery), Group IV (CCl₄ + Plant Material) and Group V (CCl₄ + silymarin). The animals were subjected to 12 hrs cycles of light and darkness. They were fed with commercially available feed pellets (12mm) containing crude protein (min 20-21 %), crude fiber (max 4 %), calcium (1-2 %) and phosphorus (0.6 %). Animals were supplied tap water from bottles during the experiment per day and the amount food and water intake is noted. [14-17]

Parameters Observed

Blood of animals was collected by cardiac puncture under light ether anesthesia during sacrifice. Blood Biochemical assays were determined using a CHEMITO SPECTRASCAN UV 2700 spectrophotometrically. The blood parameters observed were **GPT(ALT), GOT(AST), Cholesterol, Bilirubin, Triglycerides and $\sqrt{\text{GT}}$** . This was done by using Standard kits supplied by Span Diagnostics Ltd., Surat, INDIA[18-19]

Animal Grouping

Animals were grouped into five groups. Each group with 12 animals 6 males and 6 females. Reversible liver damage was induced by 0.7 ml/Kg of CCl₄ in 0.5 ml. Liquid Paraffin per animal i.p. The dose of plant powder in the form of aqueous slurry was given orally via gavages as per dose chart in table 1.

Table 1: Daily Doses Regime

Days	Group I Normal control	Group II CCl ₄ control	Group III CCl ₄ treated natural recovery	Group IV CCl ₄ + plant slurry treated	GroupV Silymarin treated
1	0.5cc liq. Paraffin & 2 cc d/w oral	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p.& 2cc d/w oral	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p. & 2cc d/w oral	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p. & 0.4 gm/kg plant slurry in 2cc d/w oral	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w oral
2	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.4 gm/kg plant slurry in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
3	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.4 gm/kg plant slurry in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
4	Sacrifice	Sacrifice	2cc d/w oral	Sacrifice	Sacrifice
5	-	-	2cc d/w oral	-	-
6	-	-	2cc d/w oral	-	-
7	-	-	Sacrifice	-	-

*All dosages are for each individual animal in the group.
The number of animals in each group 12 (6 males + 6 females).
i.p.: intraperitoneal.; d/w : Distilled Water.*

The animals from all groups were sacrificed on 4th day and for the sake of the study except the natural recovery group which was sacrificed on VIIth day after natural recovery/ regeneration of liver was initiated[20-25]

The animals were sacrificed as per the table 1. The animals were sacrificed under light ether anesthesia. The results were statistically analysed using one-way analysis of variance (ANOVA) followed by Dunnett's test for individual comparisons. $P < 0.01$ was considered significant. The blood was withdrawn from carotid artery and preserved in pre heparinized bottles which then used for further analysis.

RESULTS AND DISCUSSION

In experimental hepatopathy, the toxin (CCl_4) is biotransformed by cytochrome P450 to produce the trichloromethyl free radical. This in turn elicits lipid peroxidation of membrane lipids in the presence of oxygen generated by metabolic leakage from mitochondria. All these events culminate in loss of integrity of cell membranes and damage of hepatic tissue.

General Observations

Animals from all groups showed no abnormal behaviour in food and water consumptions. The food consumptions of animals from CCl_4 control, CCl_4 + *Leucas aspera* plant treated and CCl_4 + silymarin group decreased significantly. The CCl_4 recovery group animals showed significant decrease up to the fourth day of the treatment, and then they showed an increase. This indicates that the animals are recovering from the toxicity induced by the CCl_4 similar observations were reported with the trends in water consumption by plant material treated animals.

Biochemical parameters

CCl_4 treatment caused significant increase in plasma ALT, AST levels. The observations were competent in both the male and female animals. The plant treatment caused significant reduction in ALT and AST levels in both in male and female rats. CCl_4 treatment caused accumulation of cholesterol and the plasma levels of cholesterol were high in treated animals both in CCl_4 and CCl_4 recovery groups. *Leucas aspera* plant powder treatment significantly reduced cholesterol in all rats. Plasma levels of bilirubin significantly increased after treatment, in CCl_4 control group and CCl_4 recovery groups the levels were marginally reduced for group IV and V. Plasma levels of triglycerides increased significantly after CCl_4 treatment. These levels remain high even after natural recovery or CCl_4 treatment but *Leucas aspera* plant slurry treatment showed significant reduction in triglycerides levels in all rats.

Table 2: Blood Biochemical Parameters for all Groups

Parameter	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V
GPT(ALT)	51±2	80±4	61±2	59±2	66±5
GOT(AST)	47±5	96±2	78±3	49±7	89±3
Cholesterol	75±6	92±4	85±4	73±3	79±2
Bilirubin	0.58±0.1	0.68±0.2	0.64±0.2	0.76±0.4	0.65±0.3
Triglycerides	124±5	130±1	94±4	106±2	124±1
√GT	18±3	41±2	33±4	32±3	24±2

Plant slurry treatment caused significant reduction in cholesterol. The tissue cholesterol levels reduced after natural and Silymarin treatment. CCl_4 treatment causes classical fatty liver as

indicated by significant increase in tissue cholesterol. CCl₄ treatment significantly increased plasma gamma GT levels in all treated animals. These levels decreased after plant slurry and silymarin treatment. Assessment of liver function can be made by estimating the blood biochemical parameters were given in Table - 2

The activities of serum markers, which are enzymes originally present in higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage. The elevated levels of these marker enzymes in CCl₄-treated rats in the present study corresponded to the extensive liver damage induced by the toxin. Treatment with the test drug *Leucas aspera* as well as the standard drug silymarin significantly reduced the elevation in liver enzymes, thereby showing that *Leucas aspera* has hepatoprotective action

CONCLUSION

The present work was carried out to investigate the hepatoprotective action of *Leucas aspera* plant material on CCl₄ induced liver damage in rats. Blood biochemical assays like **GPT(ALT)**, **GOT(AST)**, **Cholesterol**, **Bilirubin**, **Triglycerides** and $\sqrt{\text{GT}}$ have been studied for evaluation of hepatoprotection. From the results of these parameters it is clear that *Leucas aspera* gave best recovery. The observations of "Group I" were matching with "Group IV" than all other groups. The combined synergistic effect of its constituents and micronutrients rather than to any single factor through free radicals scavenging activity play important role in regeneration of liver cells.

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