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Hatoprotection by fresh juice of *Leucas Aspera* leaves

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ABSTRACT

The aim of the present work is to evaluate the effect of Leucas aspera leaves fresh juice against carbon tetrachloride (CCl_4) induced liver damage. The evaluation markers used were GOT, GPT, Alkaline phosphate, glucose, bilirubin, cholesterol and total protein. These biochemical parameters were significantly changed due to single dose of CCl_4, but the treatment of Leucas aspera leaves fresh juice significantly recovers all markers to normal levels. In this study silymarin was used as a standard for comparison. The observation of markers as well as Light and electron microscope photographs supports the regeneration of liver parenchyma. This proves overall promising effect against liver disorders.

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 include 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

The leaves of *Leucas aspera* plant were collected from Avasari Forest Park, Pune, washed thoroughly and the fresh juice of leaves of *Leucas aspera* were obtained and used for toxicity study, while the hepatoprotective study was carried out in adult male and female 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 fresh juice of leaves of *Leucas aspera* with doses 2, 4 and 6ml/kg with water. 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, 10, 11, 12]

One-tenth of the maximum tested dose (i.e., 0.4 ml/kg) of the fresh juice of *Leucas aspera* leaves 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 (CCl_4 control), Group III (CCl_4 Natural Recovery), Group IV (CCl_4 + the fresh juice of *Leucas aspera* leaves) and Group V (CCl_4 + 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, 15, 16, 17]

Table I: Daily Doses Regime

DAYS	Group I Normal control	Group II CCl_4. control	Group III CCl_4 treated natural recovery	Group lV CCl_4 + plant 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 ml/kg fresh juive of lvs in 2cc d/w orally	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 ml/kg fresh juive of lvs in 2cc d/w orally	0.007gm/kg Silymarin in 2cc d/w oral
3	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.4 ml/kg fresh juive of lvs in 2cc d/w orally	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.

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 √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. The reversible liver damage was induced by 0.7 ml/Kg of CCl_4 in 0.5 ml. Liquid Paraffin per animal i.p. The dose of fresh juice of leaves of this plant in the form of aqueous slurry was given orally via gavages as per dose chart in table I.[20, 21, 22, 25]

The animals from all groups were sacrificed on 4^{th} day and for the sake of the study except the natural recovery group which was sacrificed on VII^{th} day after natural recovery of liver was initiated[24, 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.[8]

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+ the fresh juice of *Leucas aspera* leaves 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. The fresh juice of *Leucas aspera* leaves 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 the fresh juice of *Leucas aspera* leaves treatment showed significant reduction in triglycerides levels in all rats.

Plant material 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. CCl_4 treatment significantly increased plasma gama 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 - II

Table II: 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	54±2	66±5
GOT(AST)	47± 5	96±2	78±3	48±7	89±3
Cholesterol	75±6	92±4	85±4	74±3	79±2
Bilirubin	0.58±0.1	0.68± 0.2	0.64±0.2	0.56±0.4	0.65±0.3
Triglecerides	124±5	130±1	94±4	116±2	124±1
√GT	18±3	41±2	33±4	22±3	24±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 CCl_4-treated rats in the present study corresponded to the extensive liver damage induced by the toxin. Treatment with the test drug in the form of juice of *Leucas aspera* leaves as well as the standard drug silymarin significantly reduced the elevation in liver enzymes, thereby showing that the juice of *Leucas aspera* leaves has hepatoprotective action

CONCLUSIONS

The present work was carried out to investigate the hepatoprotective action of the fresh juice of *Leucas aspera* leaves on CCl_4 induced liver damage in rats. Blood biochemical assays like **GPT(ALT), GOT(AST), Cholesterol, Bilirubin, Triglecerides and √GT** have been studied for evaluation of hepatoprotection. From the results of these parameters it is clear that the fresh juice of *Leucas aspera* leaves gave best recovery. The observations of “Group I” were strongly 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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