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Short Communication

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Effect of *Lawsonia alba* leaf extracts on carbon tetrachloride-induced hepatic damage in albino rats

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Abstract

Objectives: To screen the hepatoprotective activity of leaves of *Lawsonia alba* against carbon tetrachloride induced hepatotoxicity in albino rats. Method: Studies on the hepatoprotective activity of the leaves of *Lawsonia alba* were carried out in albino rats. The *Lawsonia alba* leaf ethanolic extract and three different fractions obtained from it were screened for hepatoprotective activity using carbon tetrachloride-induced hepatic damage model in rats. Doses for the different fractions and ethanolic extract were selected based on the results of acute toxicity studies in mice. The effect was assessed by serum enzyme profile, viz., glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT) levels, and histopathological changes in liver. Results: *Lawsonia alba* ethanolic extract significantly protected from biochemical and histological changes induced by carbontetrachloride in rats. Conclusion: Ethanolic extract of *Lawsonia alba* leaves exhibited a significant protection against carbon tetrachloride-induced hepatic damage in rats.

Keywords: Carbon tetrachloride, Hepatoprotective activity, Leaves of *Lawsonia alba* Lam. (Lythraceae), Liv.52 syrup, Olive oil, 5% Gum acacia, SGOT, SGPT.

1. Introduction

Lawsonia alba Lam (Henna) of family Lythraceae is native of Indian subcontinent. It is grown as hedges in garden. Leaves of this plant are traditionally reported as a curative for various ailments like inflammation, bacterial and fungal infections, cancer, jaundice and other liver complaints. [1,2] The present study is an attempt to validate the antihepatotoxic activity of *L. alba* leaves.

Hepatotoxicity induced with CCl_4 in rats is commonly used as a model to study hepatic injury [3,4]. The involvement of free radical mediated reactions in the development of CCl_4-induced hepatic injury has been observed in

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various *in vivo* and *in vitro* studies. [5] The present study also exploits the same model for the evaluation of antihepatotoxic activity of *L. alba* leaves.

2. Materials and methods

2.1 Plant Material

The leaves (3 kg) of *Lawsonia alba* were collected from the local areas of Belgaum and botanically identified by Prof. Dr. R.C.Mathad, Dept. of Dravyaguna Vignanam, B.M.K. Ayurvedic Medical College, Belgaum, Karnataka. A voucher specimen was deposited in the Department of Pharmacognosy, K.L.E.S's College of Pharmacy, Belgaum, Karnataka, India.

The dried leaves, reduced to 60 mesh powder, was extracted with 95% ethanol under reflux. The concentrated ethanolic extract was subjected to fractionation by using petroleum ether (40-60°), butanol and butanone in succession. The extract and fractions were dried at 50°C and used for all experimental studies. The vehicle was 5% w/v acacia mucilage (1ml/kg p.o.).

2.2 Chemicals

Liv-52 syrup (The Himalaya Drug Company, Bangalore, India), Carbon tetrachloride, (E. Merck (I) Ltd., Bombay), Olive oil (Olio Sasso, Italy).

2.3 Animals

Wistar albino rats (150-200 g) and Swiss albino mice (25-30 g) of either sex used in the study were procured from experimental animal house, Dept. of Livestock Production, Govt. Veterinary College, Hebbal, Bangalore, India. They were maintained under standard husbandry conditions. The animals were given standardized laboratory feed and water *ad libitum.*

2.4 Acute Toxicity Studies

All the extracts were administered orally to different groups of mice in doses ranging from 200-3000 mg/kg. There was no mortality in any of the group. Mice which received any of the extract in doses above 2000 mg/kg, exhibited ptosis (dropping of upper eyelids) and were found to be lethargic. 1/10th of the maximum dose of the extracts tested for acute toxicity was selected for evaluation of antihepatotoxic activity i.e., 300 mg/kg p.o.[6]

2.5 Grouping of animals for the experiment

Rats were randomly divided into seven groups of six rats each.

The treatment schedule was as follows;

Group 1 – (Control) – Normal rats; 5% Gum acacia (1ml/kg, p.o., 4 days), Olive oil (2 ml/kg, S.C, 2nd and 3rd day).

Group 2 – CCl_4 treated rats; 5% Gum acacia (1ml/kg, p.o., 4 days), CCl_4 in olive oil (1:1) (2 ml/kg, S.C, 2nd and 3rd day).

Group 3 – Ethanolic extract of leaves of *Lawsonia alba* (300mg/kg, p.o., 4 days), CCl_4 in olive oil (1:1) (2 ml/kg, S.C, 2nd and 3rd day).

Group 4 – Petroleum ether extract of leaves of *Lawsonia alba* (300mg/kg, p.o., 4 days), CCl_4 in olive oil (1:1) (2 ml/kg, S.C, 2nd and 3rd day).

Group 5 – Butanol extract of leaves of *Lawsonia alba* (300mg/kg, p.o., 4 days), CCl_4 in olive oil (1:1) (2 ml/kg, S.C, 2nd and 3rd day).

Group 6 – Butanone extract of leaves of *Lawsonia alba* (300mg/kg, p.o., 4 days), CCl_4 in olive oil (1:1) (2 ml/kg, S.C, 2nd and 3rd day).

Group 7 – Standard drug treated rats (Liv-52 syrup). 5ml/kg p.o., 4 days. CCl_4 in olive oil (1:1) (2 ml/kg, S.C, 2nd and 3rd day).

The rats were sacrificed on the 5th day under light ether anaesthetic. Blood collected from

the carotid artery was allowed to coagulate at 37°C for 30 min and the serum was separated by centrifugation at 2,500 rpm and analysed for biochemical investigations i.e. SGOT and SGPT. [7] Liver was processed immediately after removal for histological investigations.

2.6 Statistical Analysis

Results of biochemical estimations were reported as mean ± S.D. for determination of significant intergroup differences, each parameter was analysed separately, and a one-way analysis of variance (ANOVA) carried out [8]. Dunnett's test (1964) was used for individual comparisons [9].

3. Results

The different fractions and ethanolic extract of *Lawsonia alba* leaves did not show any mortality up to a dose level of 3000 mg/kg, p.o. 1/10th of the maximum dose of the extract used for testing LD_{50} were chosen for the present study i.e. 300mg/kg.

The ethanolic extract of *Lawsonia alba* leaves showed marked decrease in SGOT and SGPT. The effect was comparable to Liv. 52 (Table 1). Histopathologial profile of the liver of CCl_4 treated rats showed intense centrilobular necrosis, fatty degeneration and vacuolization.

Liver of rats treated with ethanolic extract of leaves of *Lawsonia alba* significantly ameliorated the CCl_4-induced liver injury as was evident from the presence of normal hepatic cords, absence of necrosis and less degree of infiltration. Liver of rats treated with the successive fractions showed signs of protection against CCl_4 injury to some extent but it was not comparable with standard drug Liv-52.

4. Discussion

Hepatotoxicity induced by CCl_4 is attributed to generation of trichloromethyl free radicals during metabolism by hepatic microsomes, which in turn cause peroxidation of membrane lipids. Biochemical and histopathological observations clearly indicate that ethanolic extract of *Lawsonia alba* leaves exhibit significant hepatoprotective activity. Hepatoprotective action of *Lawsonia alba* is likely to be due to its ability to induce microsomal enzymes, thereby accelerating the excretion of CCl_4, or could be due to inhibition of lipid peroxidation induced by CCl_4 [10].

Table 1
Effect of the extracts of *Lawsonia alba* leaves on hepatotoxicity induced by CCl_4.

Group (n)	Biochemical parameters Mean ± S.D.	
	SGOT IU/L	SGPT IU/L
Control (6)	127.66 ± 12.37	68.33 ± 7.44
CCl_4 treated (6)	328.66 ± 53.62	274.16 ± 23.37
Ethanol extract (6)	157.00 ± 17.23*	95.5 ± 7.34*
Petroleum ether extract (6)	209.5 ± 25.44	220.83 ± 29.79
Butanol extract (6)	193.16 ± 9.98	199.16 ± 9.17
Butanone extract (6)	206.33 ± 10.19	206.66 ± 18.29
Standard drug Liv-52 (6)	146.33 ± 17.5*	86.5 ± 10.89*

Dunnett's test*, $P<0.001$ indicates Highly significant.
n= number of animals used.

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