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Hepatoprotective Activity of *Ficus mollis (Vahl)* leaf extract on CCl_4 Induced Hepatic Damage in Wistar albino rats

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ABSTRACT

Shade dried leaves of *Ficus mollis (Vahl)* were extracted using Petroleum ether (60-80^0) and investigated for the hepatoprotective activity. The activity was tested in wistar albino rats at dose level of 250mg/kg, orally and compared with silymarin (100mg/kg) as standard. At the end of the study period the rats were sacrified and serum biomarkers like ALT, AST, ALP, Bilirubin and total protein levels are measured in CCl_4 induced hepatotoxicity in rats and hepatic tissues are subjected to histopathological studies. There was significant reversal of biochemical, histological changes induced by CCl_4 treatment in rats by petroleum ether extract treatment, indicating that *Ficus mollis (Vahl)* has a significant Hepatoprotective effect.

Keywords*: Ficus mollis (Vahl)*, CCl_4, Hepatoprotective.

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INTRODUCTION

Many diseases of the liver are accompanied by jaundice caused by increased levels of bilirubin in the system. The bilirubin results from the breakup of the heamoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile.The carbon tetrachloride(CCl_4) induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Liver diseases remain one of the serious health problems. In the absences of reliable liver protective drugs in allopathic medical practices. Herbs play important role in the management of various liver disorders [1]. However, in ayurveda many indigenous plants have been used as hepatoprotective agents. A number of reviews are published stating the importance of plant drugs in the diseases of liver. This study is based on the natural products responsible for repairing and healing of adversely affected liver cells. In the present study we selected a plant namely *Ficus mollis (Vahl)* belonging to the family (*Moraceae)* commonly known as soft fig. It is a fig tree found only in south india and srilanka. It is one such plant whose stem bark, leaf used traditionally for its curative property in treating diabetes, jaundice. During casual conversation with tribal people of Andhra Pradesh, it was found that they chewed the leaves of *Ficus mollis (Vahl)* to treat jaundice [2]. However, no scientific work has been carried out on the leaves of *Ficus mollis (Vahl)* to prove the hepatoprotective activity.

MATERIALS & METHODS

The leaves of *Ficus mollis (Vahl)* was collected from chittoor district, Andhra Pradesh. The plant was authenticated and voucher specimen deposited at our library. The material was air dried under shade, powdered mechanically and stored in airtight containers. About 1 kg of the powdered material was extracted with Petroleum ether (60-80^0) by soxhlet method for 72 hours. Then the extract was filtered and concentrated under reduced pressure [3].

Drug formulation

Oral suspension containing 250 mg/ml of the Petroleum ether (60-80^0) extract was prepared in 1% w/v Carboxy methyl cellulose (CMC)

Animals

Wistar albino rats (150-200g) of either sex were used in this investigation. They were maintained at standard housing conditions and fed with commercial diet (Hindustan lever ltd., Bangalore) and provided with water *ad libitum* during the experiment. The institutional animal ethical committee (IAEC) and CPCSEA/PHA/20-09 permitted the study.

Evaluation of hepatoprotective activity

The acute toxicity studies were carried out as per staircase method [4]. Fifty rats were divided into five groups of 10 each and were administered with aliquot doses of the extracts orally. Mortality was noticed and LD_{50} of the extracts was found to be 2500 mg/kg body weight. One-tenth of this dose was selected as the therapeutic dose for the evaluation [5].

Grouping of animals

The experiment design of the investigation was carried out in 4 groups with 6 animals each group in the following regimen:

Group I (Control) : Received the vehicle 1% w/v CMC at a dose of 1 ml/kg/day of p.o for 14 days.

Group II- IV (CCl_4) : Received 0.1 ml/kg/day i.p of CCl_4 (E-Merck, Mumbai, India) for 10 days [6].

Group III (Standard) : Received Silymarin (Ranbaxy Lab. Dewas) at a dose of 100 mg/kg/day p.o for 14 days.

Group IV (Test) : Received Petroleum ether extract in the dose of 250 mg/kg/day, p.o. (as per acute toxicity studies) for 14 days.

The CCl_4, Silymarin and the extracts were administered concomitantly to the respective group of animals.

Assessment of hepatoprotective activity

All the animals were killed on day 14 under light ether anaesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37^0 C. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function viz., total bilirubin [7], total protein [8]' serum transaminases [9] and serum alkaline phosphatase [10]. These results were tabulated in Table-1.

Histopathology

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5mm thickness, processed in alcohol- xylene series and were stained with alum hematoxylin and eosin [11]. The sections were examined microscopically for histopathological changes.

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RESULTS

The administration of CCl_4 to the animals resulted in a marked increase in serum levels of ALT, AST, ALP, and bilirubin activities. However, the serum total protein level was decreased. The toxic effect of CCl_4 was controlled in the animals treated with the petroleum ether extract of *Ficus mollis (Vahl)* by way of restoration of the levels of the liver function biochemistry similar to that of standard drug Silymarin (Table 1). Histological profile of control animals showed normal hepatocytes (Fig-1). Group II animals exhibit intense centrilobular necrosis, vacuolization and macro vesicular fatty change (Fig-2).The sections of liver taken from the animals treated with standard drug Silymarin showed the hepatic architecture, which was similar to that of control (Fig-3).The animals treated with petroleum ether extract exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration (Fig-4).

Table 1: Effect of petroleum ether extract of *Ficus mollis (Vahl)* on biochemical parameters against CCl_4 - induced hepatotoxicity in rats

Group	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	Bilirubin	Protein
Control	51.07 ± 0.60	148.07 ± 0.34	173.78 ± 2.46	0.47 ± 0.02	9.23 ± 0.10
CCl_4 treated	1342.30 ± 28.27*	2155.28 ± 42.61*	404.15 ± 14.78*	2.22 ± 0.11*	6.07 ± 0.30*
CCl_4+Silymarin	87.04 ±0.31**	203.01 ± 0.97**	180.63 ± 0.56**	0.50 ± 0.01**	8.72 ± 0.01**
CCl_4 +petroleum ether extract	145.50 ± 0.71**	223.16 ± 0.83**	192.32 ± 0.75**	0.65 ± 0.03**	8.15 ± 0.02**
One-way ANOVA	F 1890.5	2083.5	151.54	164.45	58.62
	d, f 4, 25	4, 25	4, 25	4, 25	4, 25
	P<0.01	<0.01	<0.01	<0.01	<0.01

Values are expressed as mean ± SEM of six samples
* = when compared to control group
** = when compared to CCl_4 treated group
* represents *P*<0.01
Data were analyzed by One-way ANOVA followed by Tukey's test

DISCUSSION

Carbon tetrachloride, a widely used experimental hepatotoxicant, is biotransformed by cytochrome P - 450 systems to produce the trichloromethyl free radical ($CCl_3\cdot$) that causes lipid peroxidation and, thereby, produce liver damage [12]. The mechanism of the action of carbon tetrachloride is complex, multifactorial and not completely understood. When administered, carbon tetrachloride accumulates in hepatic parenchymal cells, which is metabolized to free radical $CCl_3\cdot$. The free radicals react with molecular oxygen to produce peroxy radicals (H_2O_2, O_2 and ·OH due to incomplete reduction of molecular oxygen), thereby causing oxidative

destruction of polyunsaturated fatty acids[13]. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum, rich in polyunsaturated fatty acids. Lipid peroxidative degradation of biomembrane is one of the principle causes of hepatotoxicity [14]. This is evidenced by an elevation of the serum marker enzymes ALT, AST, ALP, in the carbon tetrachloride treated rats[15][16]. When liver cell plasma membrane is damaged, a variety of enzymes, normally located in the cytosol, are released into the blood and their estimation is a useful quantitative marker of the extent and type of hepatic cell damage [17]. Administration of petroleum ether extract of *Ficus mollis (Vahl)* showed significant hepatoprotective activity, which was comparable with standard drug Silymarin. It is concluded that the petroleum ether extract of *Ficus mollis (vahl)*, at an oral dose 250mg/kg/day, is effective against the hepatotoxicity caused by CCl_4. Further profound studies are required to establish the therapeutic potential and safety of the drug of herbal origin in the treatment of hepatotoxicity.

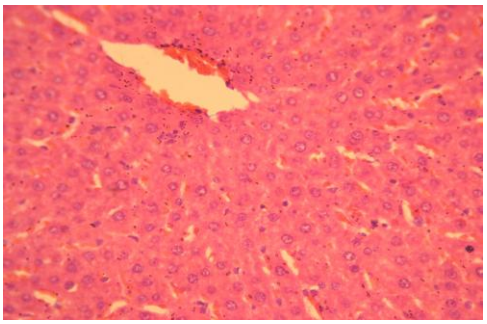

Fig-1 Section of the liver tissue of control rats showing normal histology, central vein surrounded by cords of hepatocytes.

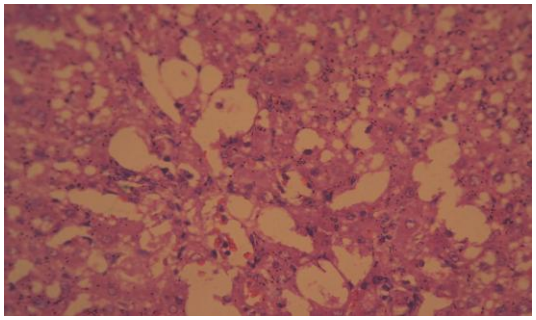

Fig-2 section of the liver tissue of rats treated with CCl_4 showing large fatty reticular background of hepatic tissue and appears vacuolated hepatocytes

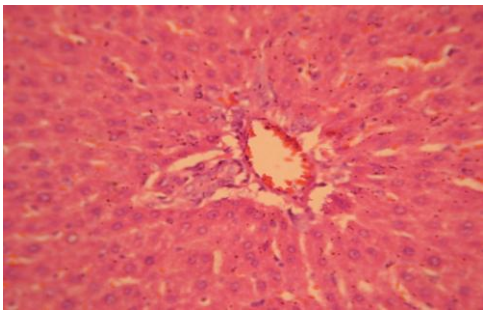

Fig-3 section of the liver tissue of Silymarin treated rats showing normal hepatocytes around central vein against CCl_4 induced hepatotoxicity hepatotoxicity

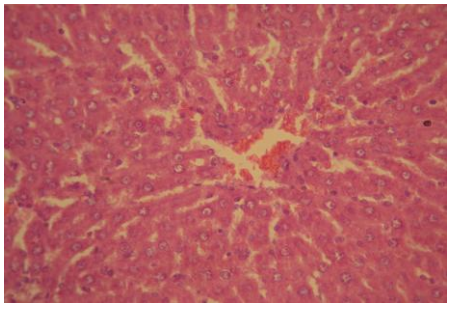

Fig-4 section of the liver tissue of petroleum ether extract treated rats showing normal hepatocytes around central vein with minute vacuolation against CCl_4 induced

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CONCLUSION

In conclusion, our study demonstrates that the leaf extract of *Ficus mollis(vahl)* can be effective in treatment against liver injury.

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