

## Hepatoprotective Activity of *Fumaria officinalis* against CCl<sub>4</sub>-induced Liver Damage in Rats

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**Abstract: Background:** Hepatoprotective activity of *Fumaria officinalis* of ethanolic extract were studied using carbon tetrachloride (CCl<sub>4</sub>) induced liver damage in rats. Liver damage was achieved by injecting 0.8 mL kg<sup>-1</sup>, s.c. of CCl<sub>4</sub> in diluted with 30% solution of liquid paraffin. **Result:** The ethanolic extract at a dose of 200 and 500 mg kg<sup>-1</sup>, p.o. offered significant (p<0.001) hepatoprotective action by reducing the serum marker enzymes like SGPT, SGOT, ALP. They also reduced the elevated levels of serum total and direct bilirubin, cholesterol, triglycerides. Ascorbic acid estimation in rat's urine and histopathological studies further conform the hepatoprotective activity of *Fumaria officinalis* when compared to the CCl<sub>4</sub> treated control groups. The results obtained were compared with Silymarin (100 mg kg<sup>-1</sup>, p.o.), the standard drug. **Conclusion:** In conclusion, ethanolic extract of *Fumaria officinalis* showed significant hepatoprotective activity.

**Key words:** CCl<sub>4</sub>-induced liver damage, *Fumaria officinalis*, hepatoprotective, urine ascorbic acid

### INTRODUCTION

Liver disease is a worldwide problem. Conventional drugs used in the treatment of liver diseases are some times inadequate and can have serious adverse effects. It is therefore, necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety. *Fumaria officinalis* (Linn.), family-Fumariceae, is a small annual herb, widely distributed in Nepal down the Nilgiri Mountains, western ghat. It is commonly known as Puttapatra in traditional medicine of Karnataka (Kirtikar and Basu, 1999; Nadkarni, 2000). *Fumaria officinalis* is important medicinal plant which is used for the treatment of various ailments in Ayurvedic system of medicine for laxative, diuretic, choleric, antispasmodic, chronic eczema and antileprotic and blood purification activity (Grieve, 1984; Launert, 1981). In addition, protopine, fumarine (Launert, 1981), benzazecin, chelanthifoline, alkaloids (Wynne *et al.*, 2004) are isolated from the plant. It also contains alkaloids (protopine, fumarine), bitter principles, mucilage, resin, fumaric acid (isomeric acid), phenols and flavonoids. The plant *Fumaria officinalis* have been reported for antispasmodic, diuretic, laxative activity (Launert, 1981). In the ethanobotanical claims, it is

mentioned as; the plant is used for the treatment of jaundice by the flock tribes (Grieve, 1984; Chopra *et al.*, 1986). But there is no scientific report on the hepatoprotective activity of *Fumaria officinalis*. Therefore, to justify the traditional claims we have assessed the hepatoprotective activity using CCl<sub>4</sub>-intoxicated rats as experimental model.

### MATERIALS AND METHODS

**Plant material:** Aerial parts of the plant of *Fumaria officinalis* were collected from the Nilgiri Hills. The plants were authenticated by Dr. Rajan (Field Botanist, Central council for research in Homeopathy, Govt. of India, Ooty). The plants were collected in the month of October.

**Preparation of the extracts:** The aerial parts of the plant were dried in shade at room temperature, powdered and this powder was packed into soxhlet column and extracted with Petroleum ether (60-80°C) for 32 h. The same marc was successively extracted with Chloroform and Ethanol for 24 h. The extracts were concentrated and dried. The yield of petroleum ether extract was 2% (w/w), chloroform extract 1.6% (w/w) and ethanolic extract 3% (w/w), respectively.

**Animals:** Wistar albino rats of either sex weighing between 150-200 g and Swiss albino mice 20-25 g were used. They were housed in standard environmental conditions and fed with rodent diet with water *ad libitum*. They were initially acclimatized for the study and the study protocol was approved by Institutional Animal Ethical Committee for the usage of animals in the experiments was obtained.

**Preliminary photochemical screening:** Tests for common photochemical were carried out by following standard methods described in Practical Pharmacognosy by Kokate *et al.* (2005).

**Behavioural effect and toxicity study:** Albino mice (Swiss strain) were divided into five groups of 10 animals were then treated with graded dose (5, 50, 300 and 2000 mg kg<sup>-1</sup>) of the extract intraperitoneally (OECD, 1993). The mice were observed continuously for 1 h for any gross behavioral changes and death, if any, intermittently for the next 6 h and then again at 24 h after dosing.

**Evaluation of hepatoprotective activity:** The rats were divided into six groups, Group I animals was maintained as negative control (Received 0.9% Normal saline, 1 mL kg<sup>-1</sup>, p.o.). Groups II-VI received Carbon tetrachloride at dose of 0.8 mL kg<sup>-1</sup> (diluted with 30% solution of liquid paraffin) i.p. for seven successive days. Group II animals were maintained as positive control, without any drug treatment. Group III, IV and V animals were treated with ethanolic extract (with a different dose of 100, 200 and 500 mg kg<sup>-1</sup>, p.o.) respectively. Group VI animals were treated with Silymarin (100 mg kg<sup>-1</sup>, p.o.) which served as standard group. The vehicle or drug treatment was carried out orally from 1st day to 7th day with concurrent administration of Carbon tetrachloride. During the period of drug treatment the rats were maintained under normal diet and water *ad libitum*. On 8th day the blood was collected by carotid artery bleeding under mild ether anesthesia (Vishwakarma and Goyal, 2004).

Serum was prepared by allowing the blood samples to coagulate on ice for 1 min followed by centrifugation (3000 rpm for 15 min) and subjected for determination of biochemical marker enzymes. Livers were removed and fixed 10% formalin solution for histopathological studies.

**Biochemical analysis:** The collected samples were used for the analysis of biochemical marker SGPT, SGOT (Rutman and Frankel, 1957), ALP (Kind and King, 1954),

bilirubin (Malloy and Evelyn, 1937), cholesterol (Deeg and Ziegenhorn, 1983) and triglycerides (Fossati and Lorenzo, 1982) levels.

**Histopathological studies:** The liver was examined grossly, stored in formalin 10% and were processed for paraffin embedding using the standard micro technique. A section of liver (5 µm) stained with aluohemotoxylin and eosin was microscopically for histopathological studies and was analyzed by a pathologist.

**Estimation of ascorbic acid:** Ascorbic acid in urine was determined by modified method developed by Roe and Kuether (1943). Wistar albino rats of either sex (150-200 g) were divided into five groups each consisting of six animals. They were kept in a metabolic cage for collection of urine. They were supplied with standard diet and water *ad libitum*, one week before and during the experimental period. Twenty four hour urine sample were collected separately for each group for one day in 5 mL of oxalic acid solution and analyzed for ascorbic acid and their average value were taken as control. Then the rats of Groups I, II, III, IV and V were treated with 0.8 mL kg<sup>-1</sup>, p.o. of CCl<sub>4</sub> respectively. Group II, III and IV were treated orally with ethanolic extract of *Fumaria officinalis* at a dose of 100, 200 and 500 mg kg<sup>-1</sup> respectively and Group V were orally treated with Silymarin 100 mg kg<sup>-1</sup>, p.o. and after one hour were challenged with CCl<sub>4</sub> (0.8 mL kg<sup>-1</sup>). The 24 h urine samples were collected at 7th day for all the groups and the samples were analyzed for ascorbic acid.

**Statistical analysis:** The results are expressed as Mean±SEM (n = 6) and were analyzed statistically using analysis of variance (ANOVA) followed by Tukey's test. Values of p<0.05 were considered significant.

## RESULTS

The ethanolic extract at a dose of 5, 50 and 300 mg kg<sup>-1</sup>, administered intraperitoneally and did not produce any mortality and a dose of 2000 mg kg<sup>-1</sup>, i.p. exhibit a sign of toxicity in mice. Hence, 1/20th, 1/10th and 1/4th of the dose i.e., 100, 200 and 500 mg kg<sup>-1</sup>, were selected for screening the hepatoprotective activity.

The results of the ethanolic extract of *Fumaria officinalis* at three dose levels (100, 200 and 500 mg kg<sup>-1</sup>, p.o.) on serum marker enzymes and total and direct bilirubin, cholesterol and triglycerides in CCl<sub>4</sub>-induced liver damage are shown in Table 1. Liver damage induced by CCl<sub>4</sub> caused significant rise in marker enzymes SGPT, SGOT, ALP and serum total bilirubin, direct bilirubin, cholesterol and triglycerides. Administration of

Table 1: Effects of ethanolic extract of *Fumaria officinalis* on Serum biochemical marker in CCl<sub>4</sub> induced liver damage in rats

Treatment	Dose ( mg kg <sup>-1</sup> )	Biochemical parameters						
		SGPT (IU L <sup>-1</sup> )	SGOT (IU L <sup>-1</sup> )	Total Bilirubin (mg dL <sup>-1</sup> )	Direct Bilirubin (mg dL <sup>-1</sup> )	ALP (IU L <sup>-1</sup> )	TGL (mg dL <sup>-1</sup> )	Cholesterol(mg dL <sup>-1</sup> )
Negative control	0.9% Normal saline(1 mL kg <sup>-1</sup> )	45.16±1.250	39.62±0.596	0.1173±0.0011	0.0274±0.0013	108.01±0.869	177.07±0.7695	196.28±1.109
Positive control (CCl <sub>4</sub> control)	0.8 mL kg <sup>-1</sup>	410.64±0.8534***	461.99±1.164***	1.2535±0.1292***	0.4407±0.0110***	842.35±0.6346***	364.61±1.257***	543.72±1.357***
Ethanolic extract t+CCl <sub>4</sub>	100	279.53±0.8567***	187.31±1.005***	0.2668±0.0013	0.1173±0.0041***	195.62±0.9544***	262.55±0.9304***	295.99±0.5020***
Ethanolic extract +CCl <sub>4</sub>	200	132.07±0.7177***	162.43±.7331***	0.2488±0.0036***	0.1450±0.0145***	145.65±1.454***	185.58±1.017***	235.00±1.470***
Ethanolic extract +CCl <sub>4</sub>	500	64.38±1.740***	65.68±1.083***	0.1440±0.0013***	0.0210±0.0004***	111.38±0.7417***	172.02±0.8448***	211.79±0.6906***
Silymarin +CCl <sub>4</sub>	100	45.78±1.515***	53.21±.5076***	0.0839±0.0012***	0.0211±0.0004***	69.23±1.0105***	168.10±1.131***	205.99±1.217***

Result are expressed as Mean±SEM (n = 6); Positive control group was compared with normal group (negative control) and values were significantly different (\*\*p<0.001); Experimental group were compared with positive control group and values were significantly different \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001

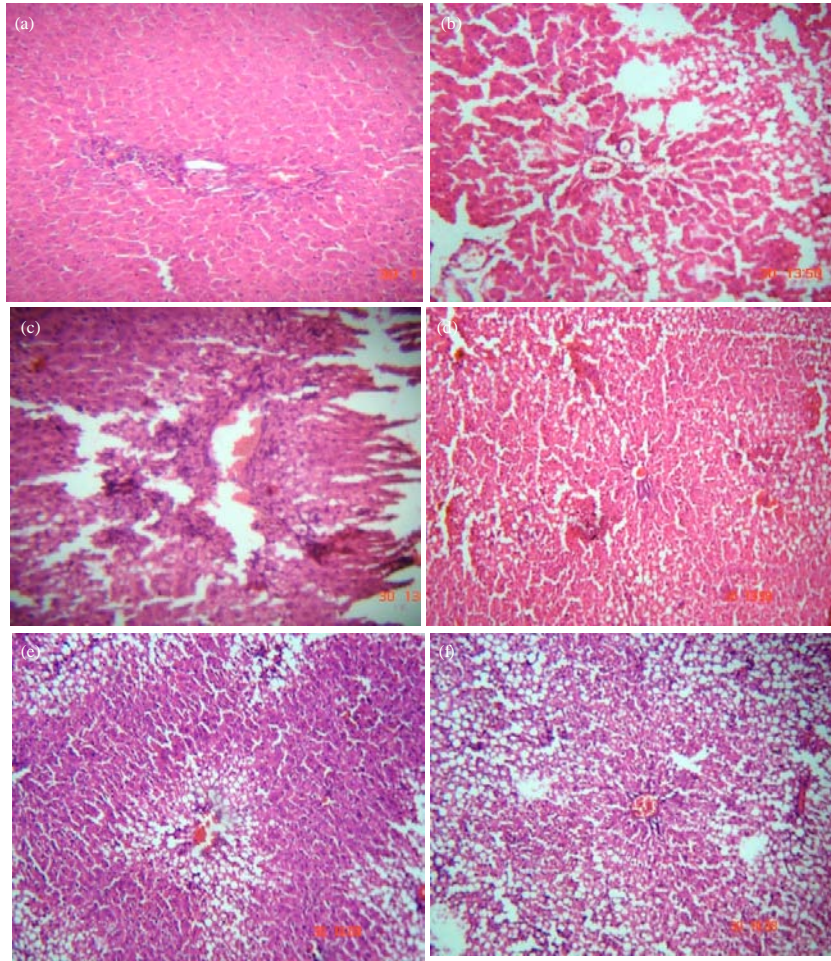


Fig. 1 (a-f): (a) Group I, liver cells of normal rats (Negative control), (b) Group II, liver cells of rats intoxicated with  $\text{CCl}_4$  (Positive control), (c) Group III, liver cells of rats treated with ethanolic extract ( $100 \text{ mg kg}^{-1}$ ) and intoxicated with  $\text{CCl}_4$  intoxicated, (d) Group IV, liver cells of rats treated with ethanolic extract ( $200 \text{ mg kg}^{-1}$ ) and intoxicated with  $\text{CCl}_4$  intoxicated, (e) Group V, liver cells of rats treated with ethanolic extract ( $500 \text{ mg kg}^{-1}$ ) and intoxicated with  $\text{CCl}_4$  and (f) Group VI, liver cells of rats treated with silymarin ( $100 \text{ mg kg}^{-1}$ ) and intoxicated with  $\text{CCl}_4$

ethanolic extract at three dose levels attenuated the increased levels of the serum enzymes, produced by  $\text{CCl}_4$  and caused a subsequent recovery towards normalization almost like that of Silymarin treatment (Table 1). From the Table 1 and 2, it is clear that extract of *Fumaria officinalis* was given orally ( $100, 200$  and  $500 \text{ mg kg}^{-1}$ ), once daily for 7 days showed dose-dependent hepatoprotective activity and highly significant effect was seen with of  $200$  and  $500 \text{ mg kg}^{-1}$  body weight, which is comparable with the standard drug, Silymarin ( $100 \text{ mg kg}^{-1}$ , p.o.). Histopathology of Group II animals ( $\text{CCl}_4$ ) showed patches of liver cell necrosis with inflammatory collections

around central vein, whereas the drug-treated groups showed absence of cell necrosis but with minimal inflammatory condition around the central vein. The extract ( $500 \text{ mg kg}^{-1}$ , p.o.) treated group showed minimal inflammatory condition with near-normal liver architecture possessing higher hepatoprotective action (Fig. 1a-f).

The daily excretion of ascorbic acid by different groups of rats before and after treatment is shown in Table 2. A dose of  $0.8 \text{ mL kg}^{-1}$   $\text{CCl}_4$  produced significant reduction in ascorbic acid excretion. Ethanolic extract of *Fumaria officinalis* delayed  $\text{CCl}_4$  induced induction in

Table 2: Effects of ethanolic extract of *Fumaria officinalis* on ascorbic acid in CCl<sub>4</sub> induced liver damage in rat's urine

Treatment	Dose mg kg <sup>-1</sup>	Mean (µg mL <sup>-1</sup> )±SEM	
		Prior to treatment	After treatment (7th day)
CCl <sub>4</sub> control	0.8 mL kg <sup>-1</sup> , s.c.	139.31±4.73	82.00±8.866
Ethanolic extract+CCl <sub>4</sub>	100	124.22±4.85	119.50±8.318* (45.73)
Ethanolic extract+CCl <sub>4</sub>	200	138.20±2.94	125.00±8.981** (52.44)
Ethanolic extract+CCl <sub>4</sub>	500	131.40±3.45	127.83±6.478** (55.89)
Standard group (Silymarin+CCl <sub>4</sub> )	100	141.22±4.22	130.83±5.695** (59.55)

Values are Mean±SEM (n = 6 in each group). Values in parenthesis are percent protection as compared to CCl<sub>4</sub> control. Experimental groups were compared with CCl<sub>4</sub> control: \*p<0.05 and \*\*p<0.01

ascorbic acid by one day prolonged treatment might have produced better benefit. Ethanolic extract of *Fumaria officinalis* and Silymarin prevented CCl<sub>4</sub> induced reduction in ascorbic acid excretion.

### DISCUSSION

It is well established that CCl<sub>4</sub> induces hepatoprototoxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl<sub>4</sub> is bio-transformed by the cytochrome P<sub>450</sub> system in the endoplasmic reticulum to produce trichloromethyl free radical (CCl<sub>3</sub>). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethylperoxyl radical, which may attack lipid on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical leads to elicit lipid peroxidation, the destruction of Ca<sup>2+</sup> homeostasis and finally, results in cell death (De Groot and Noll, 1986; Clawson, 1989; Reckengel *et al.*, 1989). These results in changes of structures of endoplasmic reticulum and other membrane, loss of enzymes metabolic enzymes activation, reduction of protein synthesis and loss of Glucose-6-Phosphatase activation, leading in liver damage (Recknagel and Glende, 1973; Azri *et al.*, 1992). Ascorbic acid is formed as a metabolite of glucose and galactose in rat liver microsomes via the glucuronic acid pathway and is excreted in urine. The enzyme UDP glucose dehydrogenase and UDP glucuronidetransferase are responsible for its formation in the liver microsomes. Its formation and excretion is altered by several drugs and substances that affect the drug metabolizing enzymes system (Satyanarayana *et al.*, 1988). The results showing reduction in ascorbic acid excretion in CCl<sub>4</sub> treated rats may reflect the inhibition of such enzymes. Alteration in urinary ascorbic acid excretion appears to be reflecting ascorbic acid in liver. Hence, the reduction in urinary ascorbic acid excretion can be used as an index for CCl<sub>4</sub> produced hepatotoxicity.

It can be concluded that, upon the literature review it is found that the aerial parts of *Fumaria officinalis*

contains protopine, fumarine, benzazecin, alkaloids, phenols, and flavonoids which are present in ethanolic extract. Therefore there is a possibility that the aerial parts of the plant may possess anti-oxidant property, which may involve in hepatoprotective property.

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