

Antihepatotoxic Effect of *Feronia Limonia* Fruit against Carbon Tetrachloride Induced Hepatic Damage in Albino Rats

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Abstract

Feronia limonia Swingle, though mentioned as a high utility medicinal plant in Ayurveda for the treatment of liver disorders, but has no scientific investigations. Therefore, the present study was undertaken to investigate the hepatoprotective activity of extracts of *Feronia limonia* fruit on albino rat liver, damage induced by single dose of carbon-tetrachloride (1.5 ml/kg, p.o.). The oral administration of extracts (500 mg/kg, p.o.) for 7 days has shown significant hepatoprotective action. There is reduction in the elevated levels of serum enzymes such SGOT, SGPT, ALP and Serum bilirubin and elevation in level of GSH, GPX, GLY and total protein in *Feronia limonia* protected rats.

Keywords: Anti-Hepatotoxic, Carbon Tetrachloride, *Feronia Limonia* Swingle

1. Introduction

Feronia limonia Swingle (Syn. *Feronia elephantum* Correa, Fam Rutaceae) are described in ayurvedic literature as Kapitha is a moderate-sized tree with straight sharp strong spines, 1.2-3.7 cm long, which is widely distributed throughout Bangladesh, Srilanka, Java and India [1-3]. The fruits of the plant are edible and considered to be a stomachic, astringent, diuretic, cardiotonic and tonic to the liver and lungs [2]; the bark is occasionally used for biliousness [4]; the leaves are aromatic and carminative and are used for the treatment of indigestion and minor bowel infections of children [2]; the roots are sometimes given for the treatment of snake-bites [2]. Previous phytochemical work was mainly focused on the isolation of coumarins [5-7], steroids and volatile components [8, 9] of the plant.

Feronia limonia has great reputation in Ayurvedic medicine for treatment of liver disorders. No scientific and methodical investigations have so far been reported in literature regarding its action on liver. Hence the present study is focused to evaluate the hepatoprotective potentials of the *Feronia limonia* fruits against CCl₄ induced liver injury in rats.

2. Experimental

2.1. Plant Collection

Fruits of *Feronia limonia* were collected during September-October 2006 from wild area in the vicinity of Allahabad. The fruit of plant was authenticated by Dr. M.P. Sharma, Reader and taxonomist, Department of Botany, Hamdard University, New Delhi. A voucher specimen (No. PD-FP-32) of plant was kept in herbarium of Hamdard University, New Delhi.

2.2. Preparation of Extract

Coarsely powdered dry fruits of *Feronia limonia* (500 g) were extracted to exhaustively with petroleum ether (60-80°C), chloroform and methanol using a soxhlet apparatus. The extracts thus obtained were dried under reduce pressure yielding 6.9%, 17.4%, 31.8% powder with reference to dry starting material respectively.

2.3. Experimental Animals

Male albino rats of wistar strain (150-200 g) were em-

ployed for assessing the antihepatotoxic activity. They were procured from the Central Animal House of Jamia Hamdard, New Delhi Sanction Letter No. (173/CPCSEA), after approval under the project proposal number-326. They were maintained under controlled condition of light (12/24 h) and temperature ($23 \pm 1^{\circ}\text{C}$). Food pellets (Hindustan lever Ltd. Mumbai, India) and tap water were provided *ad libitum*. For experimental purposes animals were kept fasting but were allowed free access to water.

2.4. CCl₄-Induced Hepatotoxicity

The animals were divided into six groups of six animals each. Group I served as normal control which received normal saline only. Group II served as CCl₄ control and received CCl₄:liquid paraffin (1:1, 1.5 ml/kg, b.w., p.o.) on first day. Group III served as standard control and received single dose of CCl₄:liquid paraffin (1:1, 1.5 ml/kg, b.w., p.o.) on first day and thereafter received treatment with standard drug silymarin (Silybon-70) (10 mg/kg, b.w., p.o.) for 7 days. Groups IV-VI received single dose of CCl₄:liquid paraffin (1:1, 1.5 ml/kg, b.w., p.o.) on first day and thereafter treated with petroleum, chloroform and methanolic extract of fruits of *Feronia limonia* (500 mg/kg, b.w., p.o.) [10], respectively, for 7 days.

2.5. Assessment of Liver Functions

Rats of all groups were anaesthetized with 1.2 g/kg b.w, of a 25% w/v aqueous solution of urethane (Loba-Chemie, Bombay), given on 8th day. The blood collected by puncturing the orbital plexus was allowed to coagulate at ambient temperature for 30 min. and the rats were sacrificed by decapitation. Serum was separated by centrifugation at 3500 rpm for 10 min. The livers of all animals were removed and processed for histological investigations.

In serum, alanine aminotransferase (ALT), aspartate aminotransferase (AST) [11], alkaline phosphatase (ALP) [12], total serum protein (TSP) [13] and bilirubin [14] were measured. Glutathione (GSH) [15], glycogen (GLY) [16] and glutathione peroxidase (GPX) [17] were estimated in liver homogenate.

2.6. Histological Investigations

Liver slices fixed for 48 h in 10% formosaline were processed for paraffin embedding following the standard microtechnique [18]. Sections (5~m) of livers stained with haematoxylin and eosin were evaluated for histopathological changes under a light microscope.

2.7. Statistical Analysis

The data were expressed as mean \pm S.E.M. (n = 6). Re-

sults were analyzed statistically by one-way ANOVA followed by Dunnett's test. The difference was considered significant if $p < 0.05$.

3. Results and Discussion

3.1. CCl₄ Induced Hepatotoxicity

It is well established from the earlier studies that the cleavage of carbon-chlorine bond (C-Cl bond) of carbon tetrachloride leads to the formation of trichloromethyl peroxy radical (CCl₃[•]O²⁻), which is involved in the pathogenesis of liver injury [19]. The abnormal higher levels of serum SGPT, SGOT, ALP and bilirubin; and decrease in total protein observed (**Table 1**) was the consequence of carbon tetrachloride induced liver dysfunction and denotes the damage to the hepatic cells [20]. Feeding of *Feronia limonia* extracts to such carbon tetrachloride-treated rats reduced the enhanced level of serum glutamate oxaloacetate transaminase (SGOT) by 16.74, 32.18, and 42.20%, serum glutamate pyruvate transaminase (SGPT) by 14.46, 31.87 and 34.12%, alkaline phosphatase (ALP) by 20.71, 25.98 and 29.64% and serum bilirubin by 39.38, 40.92, 42.19% which seems to offer the protection and maintain the functional integrity of hepatic cells. Glutathione (GSH) constitutes the first line of defense against free radicals. Reduction in liver GSH and decrease in GPX activity in carbon tetrachloride-treated rats (**Table 2**) indicated the damage to the hepatic cells, which was confirmed by the earlier reports [20]. Administration of *Feronia limonia* promoted the conversion of GSSG (oxidised glutathione) into GSH by the reactivation of hepatic glutathione reductase enzyme in carbon tetrachloride-treated animals. The availability of sufficient amount of GSH thus increased the detoxification of active metabolites of carbon tetrachloride through the involvement of GPX. But the restoration of liver glutathione (GSH) by 13.82, 37.50 and 59.04% and liver glutathione peroxidase (GPX) by 18.71, 21.37 and 39.83% after the endurance of the *Feronia limonia* extracts to such carbon tetrachloride-treated rats account for the protective efficacy of the extract. Decrease in total serum protein (TSP) (**Table 1**) and liver glycogen (GLY) (**Table 2**) observed in the rats treated with carbon tetrachloride may be associated with the decrease in the number of hepatocytes, which in turn may result into the decreased hepatic capacity to synthesize protein and GLY and consequently decrease in the liver weight (**Table 2**) but when the *Feronia limonia* extracts were given along with the carbon tetrachloride, the significant increase in the glycogen (GLY) by 31.61, 15.92 and 39.94%, total serum albumin (TSA) by 37.76%, 53.64%, 63.51% and total serum protein (TSP) by 40.83, 45.41 and 50.41 % was observed indicating the antihepatotoxic activity of the extracts and also accounting for the increase

Table 1. Effect of *Feronia limonia* extracts on the level of serum SGOT, SGPT, ALP, bilirubin and total protein.

Group	SGOT (unit/ml)	SGPT (unit/ml)	ALP (unit/ml)	Total Bilirubin (g/dl)	Total albumin (g/dl)	Total Protein (g/dl)
Group I (Normal)	59.49 ± 2.40**	48.22 ± 2.87**	47.71 ± 1.62**	2.12 ± 0.20**	4.57 ± 0.28**	7.47 ± 0.32**
Group II (CCl ₄)	175.54 ± 3.80	134.94 ± 5.02	82.06 ± 2.07	3.91 ± 0.07	2.33 ± 0.21	4.80 ± 0.25
Group III (Silymarin)	95.14 ± 3.27** (45.80%)	76.00 ± 3.04** (43.67%)	51.45 ± 1.44** (37.96%)	2.23 ± 0.18** (42.96%)	3.58 ± 0.15** (53.64%)	7.44 ± 0.18** (55.16%)
Group IV (Petroleum ether)	146.15 ± 2.46** (16.74%)	115.42 ± 4.54** (14.46%)	65.06 ± 4.42** (20.71%)	2.39 ± 0.12** (39.38%)	3.21 ± 0.19* (37.76%)	6.76 ± 0.25* (40.83%)
Group V (Chloroform)	119.05 ± 2.94** (32.18%)	91.93 ± 2.57** (31.87%)	60.74 ± 2.36** (25.98%)	2.31 ± 0.18** (40.92%)	3.58 ± 0.14** (53.64%)	6.98 ± 0.21** (45.41%)
Group VI (Methanol)	101.45 ± 2.70** (42.20%)	88.89 ± 2.91** (34.12%)	57.73 ± 1.53** (29.64%)	2.24 ± 0.23* (42.19%)	3.81 ± 0.17** (63.51%)	7.23 ± 0.17** (50.41%)

Values are mean ± S.E. (n = 6).

*P < 0.05 as compared with Group II., **P < 0.01 as compared with Group II

Table 2. Effect of *Feronia limonia* extracts on the level of liver GSH, GPX, GLY, body weight (before and after treatment) and liver weight.

Group	GSH (g/mg protein)	GLY (mg/gm of wet tissue)	GPX (micrograms of glutathione utilized/min/mg protein)	Body weight		Liver weight (gm)
				Before treatment (gm)	After treatment (gm)	
Group I (Normal)	6.15 ± 0.25**	28.17 ± 0.33**	7.89 ± 0.02**	160.9 ± 5	175.1 ± 4	6.1 ± 0.4
Group II (CCl ₄)	3.76 ± 0.19	18.60 ± 0.20	4.97 ± 0.06	162.4 ± 2	168.3 ± 3	6.08 ± 0.7
Group III (Silymarin)	6.07 ± 0.32** (61.43%)	26.99 ± 0.65** (45.10%)	7.13 ± 0.34** (43.46%)	170.02 ± 6	186 ± 7	5.96 ± 0.4
Group IV (Petroleum ether)	4.28 ± 0.27* (13.82%)	24.48 ± 0.28** (31.61%)	5.90 ± 0.15* (18.71%)	176 ± 4	191 ± 6	6.75 ± 0.2
Group V (Chloroform)	5.17 ± 0.32* (37.50%)	21.56 ± 0.38** (15.92%)	6.05 ± 0.04** (21.73%)	165 ± 3	178 ± 5	5.80 ± 0.4
Group VI (Methanol)	5.98 ± 0.39** (59.04%)	26.03 ± 0.37** (39.94%)	6.95 ± 0.06** (39.83%)	172 ± 4	188 ± 6	6.54 ± 0.6

Values are mean ± S.E. (n = 6).

*P < 0.05 as compared with Group II., **P < 0.01 as compared with Group II

in the liver weight most probably through the hepatic cell regeneration. It was conclusively observed that the methanolic extract exhibited most significant result among all the extracts.

3.2. Histopathology

Histomorphology of the livers of the untreated control (**Figure 1(a)**), the CCl₄ treated (**Figure 1(b)**) and the petroleum, chloroform, methanolic extract of *Feronia li-*

monia plus toxin treated (**Figures 1(d, e, f)**) respectively. CCl₄ administration caused gross necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis, karyolysis and eosinophilic infiltration (**Figure 1(b)**). Treatment with the extracts of the *Feronia limonia* reversed, to a large extent, the hepatic lesions produced by CCl₄, as is obvious from the absence of eosinophilia and presence of fewer necrotic zones (**Figures 1(d, e, f)**). Further, the methanolic extract exhibited most significant recovery of hepatic cells, which is in accordance with the results obtained in biochemical parameters.

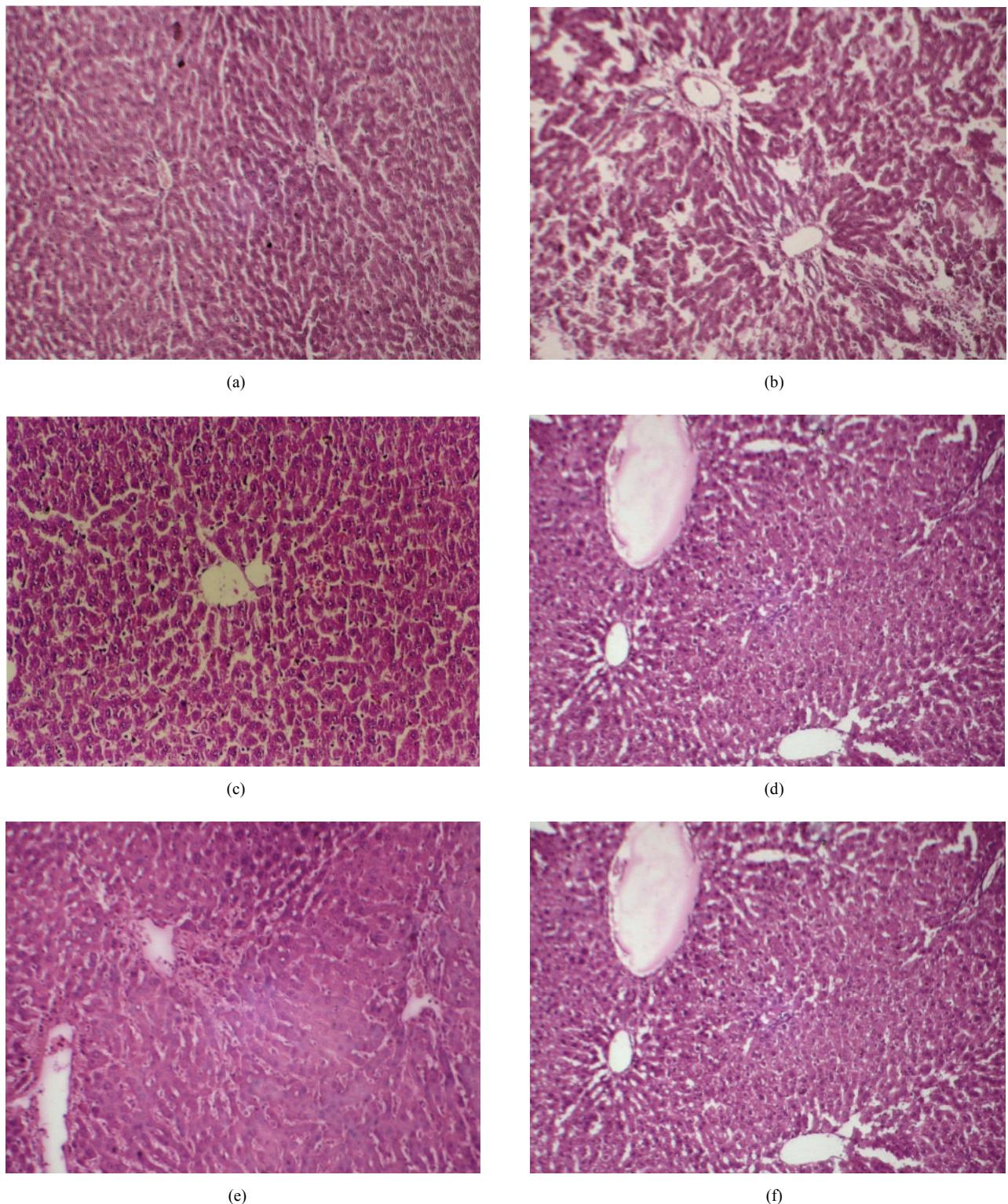


Figure 1. (a) Histology of the liver of control rat showing normal hepatic cells architecture, (b) Histology of the liver of carbon tetrachloride-treated rats showing necrosis with the obliteration of architecture in hepatic cells, (c) Histology of the liver treated with silymarin showing recovery of the hepatic cells, (d) Histology of the liver treated with petroleum extract of *Feronia limonia* showing recovery of the hepatic cells, (e) Histology of the liver treated with chloroform extract of *Feronia limonia* showing recovery of the hepatic cells, (f) Histology of the liver treated with methanol extract of *Feronia limonia* showing recovery of the hepatic cells.

4. Conclusions

It appears from our results that the mode of action of *Feronia limonia* fruit extracts in affording the hepatoprotective activity against carbon tetrachloride may be due to the cell membrane stabilization, hepatic cell regeneration and activation of antioxidative enzymes such as glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase.

5. References

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