

Protective role of standardized *Feronia limonia* stem bark methanolic extract against carbon tetrachloride induced hepatotoxicity

Mahendra Jain,^{*,****} Rakhee Kapadia,^{**} Ravirajsinh Navalsinh Jadeja,^{***}
Menaka Chanu Thounaojam,^{***} Ranjitsinh Vijaysinh Devkar,^{***} Shri Hari Mishra^{*}

^{*}Herbal Drug Technology Laboratory, ^{**}Novel Drug Delivery System Laboratory, Pharmacy Department, Faculty of Technology and Engineering, The M. S. University of Baroda, Kalabhavan, Vadodara-390001, Gujarat, India.

^{***}Division of Phytotherapeutics and Metabolic Endocrinology, Department of Zoology, Faculty of Science, The M. S. University of Baroda, Gujarat, India.

^{****}Pharmacognosy Laboratory, Faculty of Pharmaceutical Sciences, Shoolini University, Solan-173212, Himachal Pradesh, India.

ABSTRACT

Objective. This study evaluates hepatoprotective potential of *Feronia limonia* stem bark (FSB) extracts and fractions using experimental models. **Materials and methods.** Activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and cell viability were evaluated in HepG2 cells treated with carbon tetrachloride (CCl₄) in presence or absence of FL extracts or fractions. Also, plasma markers of hepatic damage, hepatic antioxidants, lipid peroxidation and histopathological alterations were assessed in rats treated with CCl₄ alone or in combination with 200 or 400 mg/kg bodyweight (BW) of FSB-7 or 25 mg/kg BW of silymarin. **Results.** *In vitro* co-supplementation of FSB extracts or fractions recorded varying degree of hepatoprotective potentials. Also, pre-supplementation of FSB methanolic extract (FSB-7) followed by CCl₄ treatment significantly prevented hepatic damage and depletion of cellular antioxidants. Also, CCl₄+ FSB-7 group showed minimal distortion in the histoarchitecture of liver and results were comparable to that of CCl₄+ silymarin treated rats. **Conclusion.** This inventory is the first scientific report on hepatoprotective potential of FSB methanolic extract.

Key words. Antioxidants. Bioassay guided fractions. Histopathology. Marmesin. Silymarin.

INTRODUCTION

Mammalian liver is a key organ associated with synthesis and secretion of digestive enzymes and elimination of toxic substances. Therefore toxicity of liver cells causes impairment of its function and associated physiological complications.¹ Infectious pathogens and hepatotoxic chemicals are the main causative agents inducing impairment of liver function.² Hence, natural remedies of herbal origin are considered to be an effective and safe alternative the-

rapy as some of the synthetic drugs manifest hepatotoxicity due to their side effects. In Ayurveda, there are a variety of herbal treatments suggested for prevention of liver disorders.³ However, it is imperative to establish their efficacy and safety prior to development of newer medicaments acting against hepatic injury.

Feronia limonia (family *Rutaceae*, subfamily *Aurantioideae*), is commonly known as “kaitha” or wood apple and is widely distributed in deciduous and arid landscapes of several countries in South Asia.⁴ *Feronia limonia* (FL) as a whole, or its parts such as unripe and ripe fruit, root, bark, trunk gum and leaves have a broad spectrum of traditionally established therapeutic potentials. Stem bark of FL has been reported to provide relief in cases of heartburns.⁵ Ethnic inhabitants of eastern India (Santals and Juangs) use stem bark extract of FL for treating asthma, bronchitis and skin ailments.^{6,7} Stem bark of FL has been repor-

Correspondence and reprint request: Mahendra Jain, M. Pharm., Ph.D.
Pharmacognosy Laboratory, Faculty of Pharmaceutical Sciences, Shoolini
University
Solan-173212, Himachal Pradesh, India. M. +919355488868
E-mail: mjainms@yahoo.com

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ted to be rich in coumarins (bergapten, psoralen, demethylsubrosein, marmesin), tetranortriterpene (acidissimin), and flavanoids (Glucopyranoside, Urosolic Acid, (-)-(2S)-5,3“Dihydroxy-4”-methoxy-6”,6”-dimethylchromeno-(7,8,2”,3”)-flavone).⁸⁻¹¹

Recent studies from our lab have reported hepatoprotective potential of FL leaf¹² and root¹³ extracts. In continuation, present study assesses the hepatoprotective potential of bioassay guided fractions of FL stem bark using *in vitro* and *in vivo* experimental models.

MATERIALS AND METHODS

Plant material

Feronia limonia stem bark was collected during September-October 2008 from campus of The Maharaja Sayajirao University of Baroda, Vadodara, India. They were authenticated in the Department Botany and a voucher specimen (No. Pharmacy/FL/08-09/01/MJ) was deposited in the Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara, India.

Preparation of extracts

The stem barks were shade dried, powdered (500 g) and extracted three times with petroleum ether (3 x 1.5 L) in a soxhlet apparatus. The filtrates were then combined, filtered and concentrated to dryness in a rotary evaporator (Buchi-R-215, Germany) to obtain a crude petroleum ether extract (FSB-1). The remaining marc was then dried and exhaustively extracted at 60-80 °C with methanol (3 x 1.5 L) in a soxhlet apparatus. The pooled extracts were then concentrated under vacuum to obtain methanolic extract (FSB-7). Hydro methanolic extract was made by addition of hot distilled water in methanol (1:1) and partitioned with chloroform (100 mL x 4) and combined chloroform fraction was then concentrated in vacuum to obtain a brown residue (4.5 g) (FSB-9). This residue was chromatographed over a Silica gel (60#120 mesh size) column and eluted with toluene followed by ascending concentrations of ethyl acetate and methanol. The resultant 7th fraction obtained from ethyl acetate- toluene (40:60) yielded an amorphous yellow powder on drying (FSB-11). The subsequent 10th fraction obtained from ethyl acetate-toluene (60:40) yielded yellow crystalline fraction (FSB-12).

Standardization of FSB-S7 using marmesin

Precisely weighed samples (*Feronia limonia* stem bark) were extracted with methanol in an ultrasonic bath and filtered in 0.22 mm filter. An aliquot of 20 µL of sample was injected onto the high-pressure liquid chromatography (HPLC) column (XTerra™ RP C18 column, 4.6 x 250 mm, 5 mm particle size) and elution was carried out with methanol: water (1:1) at a flow-rate of 2 mL/min and the eluate was monitored at 280 nm. The procedure was repeated three times for each sample. The external standard calibration curve for marmesin was prepared with calibration solution with in the concentration range of 10–50 µg/mL. Each solution was prepared and injected three times and the curve was constructed (using Microsoft Office Excel 2007). The calculated concentration of marmesin was expressed in terms of percentage w/w.

In vitro hepatoprotective studies

- **Cell culture.** Human liver hepatoma cells (HepG2) (obtained from National Centre for Cell Sciences, Pune, India) were seeded (1 x 10⁵ cells/T 25 Flask) and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antimicrobial-antimycotic solution for 24 h at 37°C with 5% CO₂ (Thermo scientific, forma II water jacketed CO₂ incubator). Cells were sub-cultured every third day by trypsinization with 0.25 % Trypsin-EDTA solution. All the reagents were sterile filtered through 0.22 micron filter (Laxbro Bio-Medical aids Pvt. Ltd, Mumbai, India) prior to use for the experiment.
- **Cytotoxicity assay.** HepG2 cells (5.0 x 10³ cells/well) were maintained in 96 well culture plate (Tarson India Pvt. Ltd) for 72 hr in presence of FSB-1, FSB-7, FSB-9, FSB-11 or FSB-12 at the concentrations of 10, 20, 100, 250, 500, 750 and 1,000 µg/mL. At the end of incubation period, 10 µL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 mg/mL) was added to wells and the plate was incubated at 37°C for 4 h. At the end of incubation, culture media was discarded and wells were washed with phosphate buffer saline (Himedia Pvt. Ltd, Mumbai, India). Later, 150 µL of dimethyl sulphoxide was added to all the wells incubated for 30 min at room temperature with constant shaking. Absorbance was read at 540 nm using ELX800 universal microplate reader (Bio-Tek instruments, Inc,

Winooski, VT) and subsequently percentage cell viability was calculated.¹⁴

- **CCL4 induced hepatotoxicity assay in HepG2 cells.** HepG2 cells (5.0×10^3 cells/well) were maintained in culture media containing 1% carbon tetrachloride (CCl₄) in presence or absence of FSB-1, FSB-7, FSB-9, FSB-11, FSB-12 or silymarin at the concentrations of 10, 20, 50, 100, 200 $\mu\text{g/mL}$ for 24 h. Later, supernatants were removed and activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined. Cell viability assay was performed in the adherent cells as per the modified method described in our previous report.¹²

Experimental animal

Male Wistar albino rats (obtained from Zydus Research Centre, Ahmedabad, India) were housed and maintained in clean polypropylene cages and fed with laboratory chow (Pranav agro ltd, India). Experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the institutional animal ethical committee (IAEC) of Department of Pharmacy, Faculty of

Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara, INDIA.

Acute toxicity studies

Acute oral toxicity study was conducted using the limit test procedure as per the organization for economic co-operation and development (OECD) test guidelines on acute oral toxicity test 401.15 Thirty two Wistar rats of either sex were divided into four groups (n = 8) and were orally administered with a single dose of 1,000 mg, 2,000 mg, 3,000 mg or 5,000 mg/kg BW of FSB-7. Animals were observed for possible behavioral changes such as tremors, convulsions, sleep, altered feeding, salivation, altered somatomotor activities and diarrhea for 7 days post treatment.

In vivo hepatoprotective studies

Thirty rats were randomly divided into 5 groups of 6 each. Group-I (CON) served as normal control and was orally given 0.5% carboxy methyl cellulose (CMC) solution daily for 7 days. Group-II (CCl₄) were given 0.5% CMC solution (0.1 mL) daily for 7 days. Group-III and IV (CCl₄+FSB-7A and CCl₄+FSB-7B) were orally given 200 or 400 mg/kg

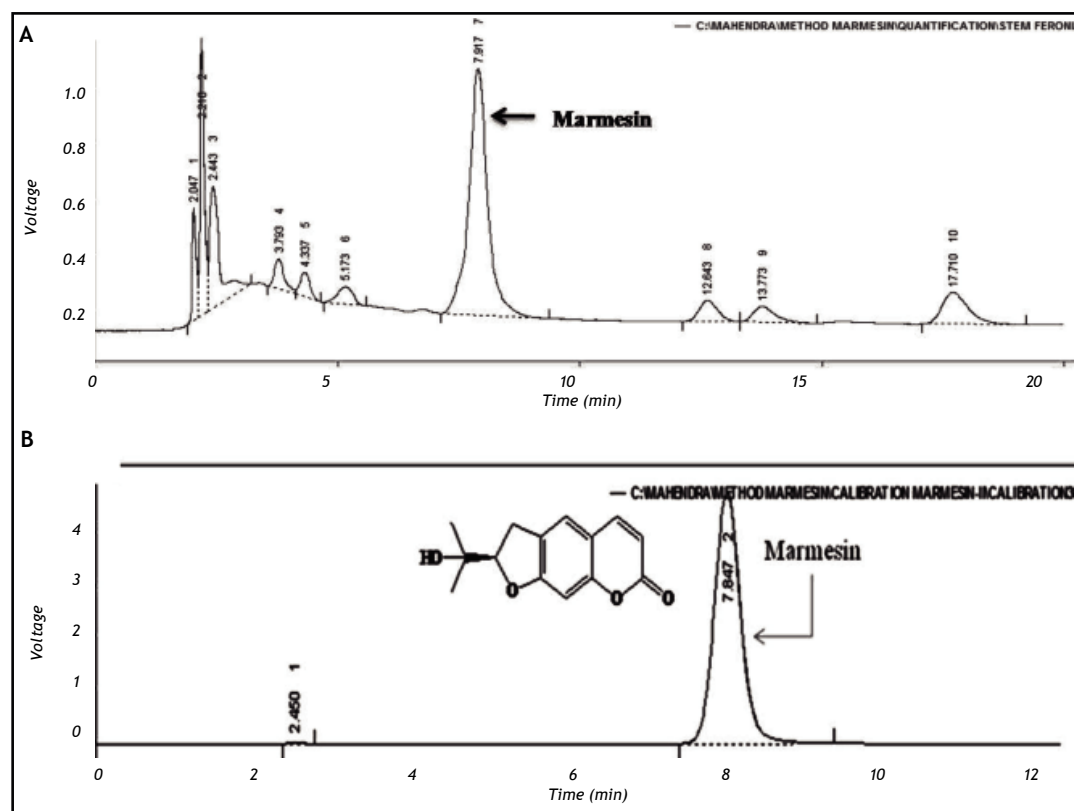


Figure 1. HPLC chromatogram of (A) methanolic extract of *Feronia limonia* stem bark showing presence of marmesin and (B) standard marmesin.

of FSB-7 once daily for 7 days respectively. Group-V (CCl₄+SYL) were orally given silymarin (25 mg/kg) once daily for 7 days.¹⁶ Groups II, III, IV and V were injected with a single dose of 0.5 mL/kg CCl₄ (i.p.) mixed with olive oil (1:1) on 7th day of the study¹⁶ whereas, group I received equal volume of olive oil.

On the 8th day, blood sample were collected via retro-orbital sinus puncture under mild ether anesthesia and plasma was separated for biochemical analysis. Later, animals were sacrificed by cervical dislocation under mild ether anesthesia and liver was excised and stored at -80 °C for further estimations.

Table 1. Effect of *Feronia limonia* extracts, fractions and silymarin on carbon tetrachloride (CCl₄) induced hepatotoxicity.

Treatments	Aspartate aminotransferase (IU/L)	Alanine aminotransferase (IU/L)	Cell viability (%)
Control	5.00 ± 0.57	4.00 ± 0.88	100.00 ± 1.00
1% CCl ₄	15.00 ± 3.46 †	10.00 ± 1.78 †	20.81 ± 1.21 †
1% CCl ₄ + Silymarin (µg/mL)			
10	6.33 ± 0.33**	5.33 ± 0.88**	74.28 ± 1.70***
20	6.33 ± 0.66**	3.33 ± 0.66***	81.17 ± 1.99***
50	4.00 ± 0.57***	2.66 ± 0.88***	84.41 ± 1.87***
100	4.00 ± 1.00***	2.66 ± 0.33***	94.54 ± 4.10***
200	3.33 ± 0.88***	2.33 ± 0.88***	96.85 ± 3.45***
1% CCl ₄ + FSB-1 (µg/mL)			
10	6.33 ± 0.66***	6.66 ± 0.88***	90.71 ± 2.53***
20	5.33 ± 0.33***	5.66 ± 0.33***	95.40 ± 0.61***
50	4.66 ± 0.33***	4.33 ± 0.33***	99.34 ± 0.72***
100	3.66 ± 0.66***	3.66 ± 0.88***	86.02 ± 1.34***
200	3.00 ± 0.57***	2.33 ± 0.33***	86.26 ± 1.25***
1% CCl ₄ + FSB-7 (µg/mL)			
10	8.00 ± 0.57*	5.00 ± 1.15***	95.82 ± 2.86***
20	5.33 ± 0.33***	6.00 ± 0.57***	96.39 ± 1.00***
50	4.33 ± 0.33***	5.66 ± 0.33***	88.18 ± 1.73***
100	2.66 ± 0.88***	2.66 ± 0.66***	91.74 ± 4.03***
200	2.33 ± 0.33***	2.33 ± 0.33***	86.96 ± 2.23***
1% CCl ₄ + FSB-9 (µg/mL)			
10	10.66 ± 1.33NS	7.00 ± 0.57*	97.74 ± 0.24***
20	8.33 ± 0.88*	6.33 ± 0.88**	83.02 ± 4.15***
50	5.00 ± 0.57***	4.66 ± 0.88***	86.16 ± 4.36***
100	3.66 ± 0.33***	3.00 ± 1.15***	81.76 ± 4.97***
200	3.66 ± 0.33***	2.33 ± 0.88***	90.62 ± 0.16***
1% CCl ₄ + FSB-11 (µg/mL)			
10	9.66 ± 1.45NS	7.66 ± 0.33NS	86.35 ± 1.48***
20	8.33 ± 0.33*	8.33 ± 0.33 NS	76.41 ± 3.44***
50	10.33 ± 0.88NS	6.60 ± 0.33 NS	71.26 ± 2.27***
100	7.33 ± 0.33*	7.33 ± 1.20 NS	67.65 ± 1.48**
200	7.66 ± 0.88*	7.00 ± 0.57 NS	52.49 ± 1.86**
1% CCl ₄ + FSB-12 (µg/mL)			
10	10.33 ± 0.33 NS	8.33 ± 0.33NS	34.40 ± 0.70*
20	9.00 ± 0.57 NS	6.00 ± 0.57**	41.52 ± 0.99*
50	8.33 ± 0.88*	6.00 ± 1.15**	67.96 ± 0.89**
100	2.66 ± 0.33***	3.33 ± 0.88***	75.64 ± 0.39***
200	4.00 ± 0.57***	4.66 ± 0.33***	52.88 ± 0.29**

Data expressed as mean ± SEM for n = 3. †p < 0.001 compared to control and *p < 0.05. **p < 0.01 and ***p < 0.001 compared to 1% CCl₄.

- **Plasma biochemical assays.** Plasma AST, ALT, alkaline phosphatase (ALP), total bilirubin and total protein were assayed using commercially available kits (Reckon diagnostics, Baroda, India).
- **Hepatic antioxidants and lipid peroxidation.** Liver of control and treated animal were excised, weighed and homogenized in chilled (4 °C) tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were then centrifuged at 10,000 x g at 0 °C for 20 min in high speed cooling centrifuge. Supernatant was used for the assay of superoxide dismutase (SOD),¹⁷ catalase (CAT),¹⁸ reduced glutathione (GSH),¹⁹ total protein²⁰ and lipid peroxidation (LPO)²¹ whereas, ascorbic acid (AA) content was determined in sediment.²²
- **Histopathological evaluations of liver.** Liver samples were fixed in 4% buffered paraformaldehyde, dehydrated in graded alcohol series and embedded in paraffin wax. Five μ m thick sections were cut and stained with hematoxyline and eosin and examined for gross structural changes. Photographs were taken using Canon power shot S72 digital Camera (200 X).

Statistical analysis

Data was analyzed for statistical significance using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test and results were expressed as mean $\pm$ SEM using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego California USA.

RESULTS

Quantitative analysis of marmesin by HPLC

The calibration curve was prepared with marmesin and was found to be linear ($R^2 = 0.988$) in the concentration range (10-50 μ g/mL). The concentration of marmesin in FSB-7 was found to be 0.03112% w/w. The chromatogram obtained for FSB-7 revealed multiple peaks including that of marmesin (Figure 1).

Cytotoxicity studies

Cytotoxicity assessment of FSB-1, FSB-7 and FSB-11 revealed an identical pattern of cytotoxicity that was marked by > 50% viable cells at 250 μ g/mL dose. However FSB-9 and FSB-12 appeared to be relatively more toxic because they showed < 50 % viability at 250 μ g/mL dose (Figure 2).

In vitro hepatoprotective studies

Activity levels of AST and ALT in cell supernatants recorded a significant increment in cells treated with 1% CCl₄. However, co-supplementation of FSB-1, FSB-7 or FSB-9 recorded a non-linear dose dependent decrease in activity levels of AST and ALT that were comparable to dose dependent decrease observed in CCl₄ + silymarin treated groups (Table 1). Similar set of results were observed in cell viability of HepG2 cells (Table 1).

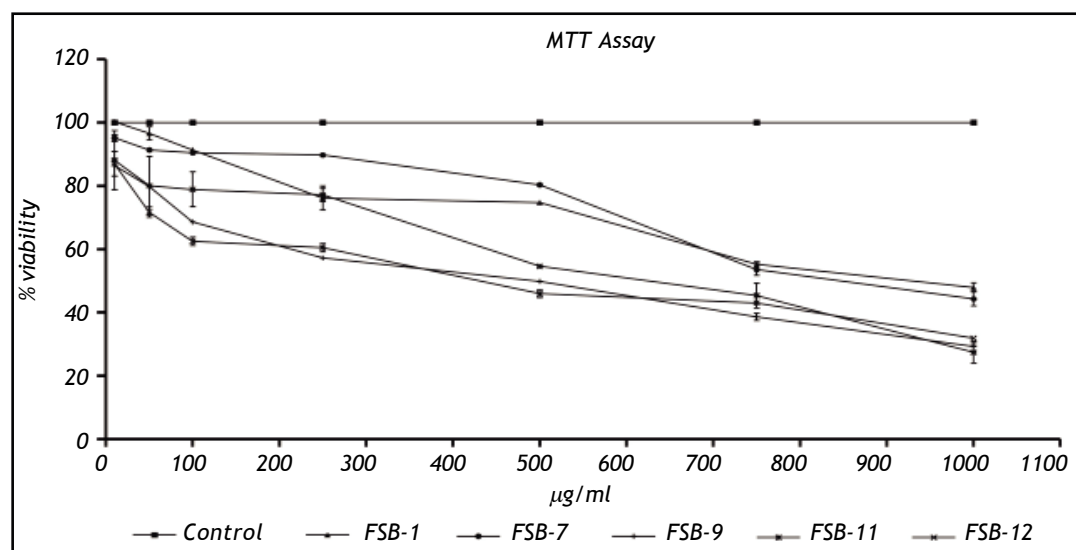


Figure 2. Cytotoxicity evaluations of *Feronia limonia* stem bark extracts and fractions. Data expressed as mean $\pm$ SEM for n = 3.

Acute toxicity studies

No mortality was recorded in animals that were orally administered up to 5,000 mg/kg of FSB-7. Also there were no adverse behavioral changes, diarrhea, salivation or food aversion. No major changes in the gross weight of organs (data not shown) could be observed following FSB-7 (5,000 mg/kg) administration.

rats. FSB-7 pre-treatment was able to prevent CCl₄ induced depletion of hepatic antioxidant. A dose of 500 mg/kg BW of FSB-7 was found to be most efficient and comparable to SYL + CCl₄ treated group. Hepatic LPO levels were significantly high in CCl₄ treated groups. But, LPO level were similar in SYL, FSB-7 treated groups and comparable to control group (Table 2).

Hepatic antioxidants and lipid peroxidation

Significant decrement in activity levels of hepatic enzymatic (SOD and CAT) and non-enzymatic (GSH and AA) antioxidants were recorded in CCl₄ treated

Plasma markers of hepatic injury

Significantly higher levels of AST, ALT, ALP and total bilirubin were recorded in CCl₄ treated rats whereas; the total protein content in plasma was significantly reduced. However, FSB-7 treated groups prevented CCl₄ induced elevation in plasma

Table 2. Effect of *Feronia limonia* stem bark methanolic extract (FSB-7) and silymarin (SYL) on hepatic antioxidants and lipid peroxidation during carbon tetrachloride (CCl₄) induced hepatotoxicity.

Experimental groups	Superoxide dismutase (U/min/mg protein)	Catalase (U/min/mg protein)	Reduced glutathione (mg/g)	Ascorbic acid (mg/g)	Lipid peroxidation (nmol of MDA formed/mg protein)
Control	62.21 ± 4.31	33.45 ± 1.56	7.00 ± 0.45	4.21 ± 0.25	2.77 ± 0.39
0.5 mL/kg CCl ₄	25.53 ± 1.88 †	16.14 ± 1.99 †	2.45 ± 0.21†	1.89 ± 0.22 †	5.67 ± 0.21†
SYL (25 mg/kg) + 0.5 mL/kg CCl ₄	52.11 ± 4.89***	31.11 ± 2.11**	4.88 ± 0.34***	3.54 ± 0.17***	2.41 ± 0.29***
FSB-7 (200 mg/kg) + 0.5 mL/kg CCl ₄	45.00 ± 4.11***	22.33 ± 0.53*	3.64 ± 0.02*	2.86 ± 0.04**	3.31 ± 0.02**
FSB-7 (400 mg/kg) + 0.5 mL/kg CCl ₄	51.00 ± 5.01***	27.00 ± 0.35**	4.23 ± 0.25***	3.23 ± 0.05***	2.92 ± 0.01***

Data expressed as mean ± SEM for n = 6. †p < 0.001 compared to control and *p < 0.05, **p < 0.01 and ***p < 0.001 compared to CCl₄.

Table 3. Effects of *Feronia limonia* stem bark methanolic extract (FSB-7) and silymarin (SYL) on plasma levels of hepatic injury markers during carbon tetrachloride (CCl₄) induced hepatotoxicity.

Treatments	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Alkaline phosphatase (U/L)	Total bilirubin (mg/dL)	Total protein (g/dL)
Control	46.11 ± 2.99	84.66 ± 9.00	1.69 ± 0.13	1.40 ± 0.22	8.00 ± 0.84
0.5 mL/kg CCl ₄	140.41 ± 4.23 †	280.16 ± 12.41†	3.78 ± 0.31†	3.88 ± 0.42†	4.54 ± 0.94†
SYL (25 mg/kg) + 0.5 mL/kg CCl ₄	52.41 ± 3.00***	92.11 ± 12.56***	1.45 ± 0.23*	1.51 ± 0.21***	7.00 ± 0.71***
FSB-7(200 mg/kg) + 0.5 mL/kg CCl ₄	71.66 ± 2.59**	142.16 ± 13.04**	2.52 ± 0.10*	2.50 ± 0.25**	6.26 ± 0.02**
FSB-7(400 mg/kg) + 0.5 mL/kg CCl ₄	62.83 ± 4.20***	121.16 ± 11.76***	2.25 ± 0.21*	2.07 ± 0.02**	6.55 ± 0.03**

Data expressed as mean ± SEM for n = 6. †p < 0.001 compared to control and *p < 0.05, **p < 0.01 and ***p < 0.001 compared to CCl₄.

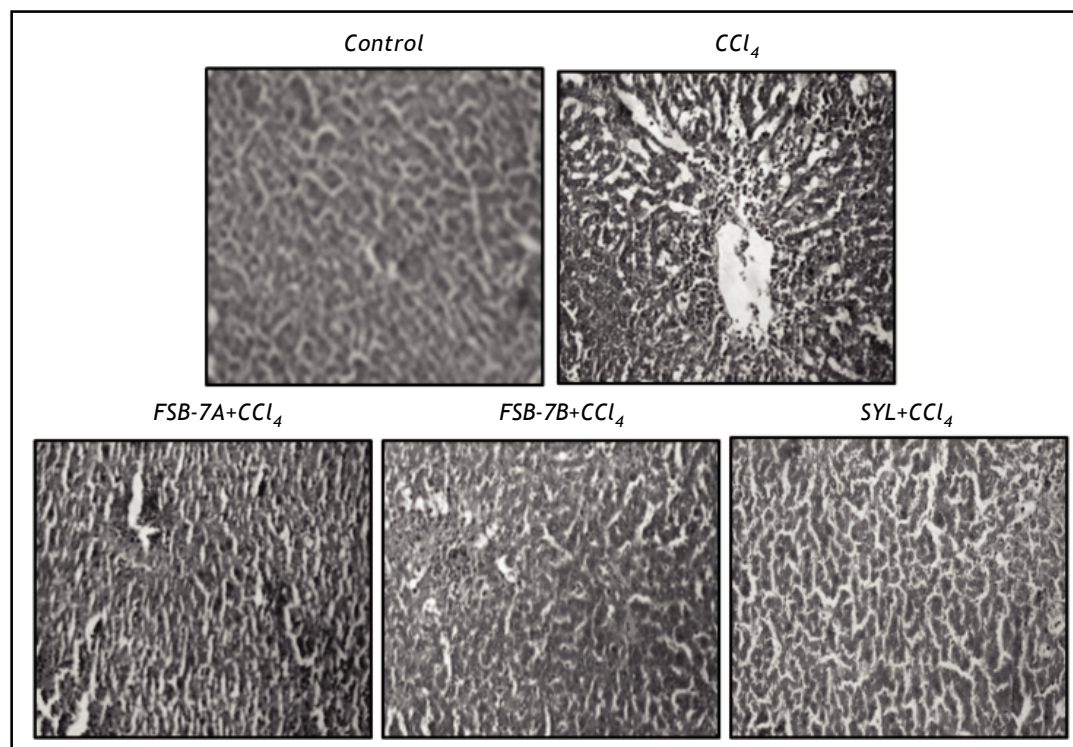


Figure 3. Haematoxyline and eosin stained photomicrographs of rat liver treated with vehicle (0.5% CMC), carbon tetrachloride (CCl_4) alone or in presence of *Feronia limonia* stem bark methanolic extract (FSB-7) or silymarin (SYL).

markers of hepatic damage and prevented CCl_4 induced decrement in plasma protein. However, FSB-7 treatment prevented CCl_4 induced changes in these parameters with 400 mg/kg BW dose being the most efficient (Table 3).

Microscopic evaluation of hepatic tissue

Hepatocyte vacuolation, centrilobular necrosis and nuclear condensation were evident in hematoxyline and eosin stained liver section of CCl_4 group (Figure 3). These cellular changes were greatly reduced in FSB-7 treated group with higher dose (400 mg/kg) recording mild necrosis and relatively healthy hepatocytes that were comparable to the control and SYL treated groups (Figure 3).

DISCUSSION

Prior to the therapeutic use of any herbal extract, it is imperative to perform a cytotoxicity assay. This is because crude extract of many herbs have been shown to be non-toxic but some of its bioassay guided fraction or pure compound may show toxicity.²³ In the present study, cytotoxicity evaluation was performed with the dose range of 10-1,000 $\mu\text{g/mL}$ of FL extracts and fractions (FSB-1, FSB-7, FSB-9, FSB-11 and FSB-12). Since, all the extract/

fractions tested for cytotoxicity assay showed > 50% cell viability at 250 $\mu\text{g/mL}$ dose, they were used for *in vitro* hepatoprotective study.

HepG2 cell line is a popular *in vitro* model where in, cells are subjected to CCl_4 induced toxicity and subsequent amelioration using a desired herbal extract or compound in question.²⁴ In the present study, various extracts/fractions of FL (FSB-1, FSB-7, FSB-9, FSB-11 and FSB-12) were subjected to scrutiny for assessing their hepatoprotective potentials. Results revealed that FSB-7 and FSB-9 imparted hepatoprotection and the same was evident in form of a dose dependent decrement in activity levels of AST and ALT and improved cell viability. Based on these observations and toxicity study, FSB-7 was chosen for subsequent *in vivo* analysis of its hepatoprotective potential.

It has been reported that, hepatic cytochrome P450 dependent monooxygenases converts CCl_4 into highly reactive trichloromethyl (CCl_3) radicals. These CCl_3 radicals then, alkylate the cellular protein and abstract an electron from polyunsaturated fatty acid of cellular membrane to initiate lipid peroxidation.¹⁶ These lipid peroxides create severe intracellular oxidative stress and depletion of cellular antioxidants resulting in gross damages to the DNA, cell membrane and mitochondria.¹⁶

The non-enzymatic antioxidant, GSH is one of the most abundant tripeptides, widely distributed in liver cells. Its functions are mainly concerned with the removal of free radical species such as hydrogen peroxides, superoxide radicals, alkoxy radicals and maintenance of membrane protein thiols.²⁵ The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD and CAT. These enzymes constitute a mutually supportive team of defense against reactive oxygen species (ROS). Superoxide dismutase represents the first line of defense against free radicals and catalyze dismutation of superoxide radical to oxygen and hydrogen peroxide.²⁶ Catalase enzyme catalyzes the decomposition of hydrogen peroxide and other peroxides (e.g., lipid peroxides in cell membranes) into oxygen and water, using reduced glutathione as substrate.²⁶ In consistence with the previous reports,^{26,27} we recorded elevated indices of LPO, and decrement in GSH, AA, SOD and CAT in CCl₄ treated rats. However, FSB-7 (400 mg/kg) was capable of preventing oxidative stress and depletion of tissue antioxidants. Presently recorded minimum depletion of antioxidants and lowered LPO indices in FSB-7 treated rats is due to high content of marmesin that scavenges free radicals produced due to CCl₄ treatment.¹²

Microscopic evaluation of liver sections of control rats depicted unaltered cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein. Whereas, liver section of CCl₄ treated rats showed loss of cellular boundaries, infiltration of inflammatory cells, cytoplasmic vaculation, fatty change, centri-lobular necrosis and severe collagen deposition. These observations corroborate other reports on hepatic damage following CCl₄ treatment. 28-29 FSB-7 supplementation to CCl₄ treated rats minimized these set of changes in a dose dependent manner and the results were comparable to that of control and CCl₄ + SYL groups. These results are in accordance with other reports on protective role of herbal extract against CCl₄ induced hepatic damage³⁰ and justifies presently recorded biochemical and microscopic changes.

Carbon tetrachloride induced hepatocyte damage or necrosis results in the leakage of marker enzymes of liver function and hence, elevations in plasma levels of AST, ALT, ALP, bilirubin and total protein are indicators of hepatotoxicity.³¹ Presently recorded elevated levels of AST, ALT, ALP, bilirubin and total protein in the CCl₄ treated rats are in accordance with previous reports.^{32,33} The restora-

tion of these parameters to near normal levels in CCl₄+FSB-7 treated group indicates at the ability of FSB-7 in preventing cellular damage and subsequent leakage of intracellular enzymes and bilirubin. The same stands well correlated with histopathological evaluations. It is assumed that FSB-7 imparts hepatoprotection against free radicals induced oxidative damage and stabilizes the hepatic cells against further degradation.

CONCLUSION

This study establishes the hepatoprotective potential of FL stem bark and the same is attributable to the presence of marmesin in FSB-7. It may be required a detailed investigation at the clinical level to translate the present findings into developing a new hepatoprotective herbal medicine.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

REFERENCES

1. Mroueh M, Saab Y, Rizkallah R. Hepatoprotective activity of Centaurea erythraea on acetaminophen-induced hepatotoxicity in rats. *Phytother Res* 2004; 8: 431-3.
2. Navarro V, Senior J. Drug-related hepatotoxicity. *New Eng J Med* 2006; 354: 731-9.
3. Chatterjee TK. Herbal Options. Kolkata, INDIA: Books and Allied Ltd; 2000.
4. Chopra AS. "Āyurveda" in Selin, Helaine. In: Medicine across cultures: History and practice of medicine in Non-Western cultures. Norwell, MA: Kluwer Academic Publishers, 2003, p. 75-83.
5. Warriar PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plant: A compendium of 500 species. Orient longman limited Andhra Pradesh, India; 1994, p. 327-32.
6. Panda H. Medicinal plants cultivation and their uses. Asia Pacific Business Press, Inc. India; 2000, p. 301-2.
7. Bakshi DNG, Sensarma P, Pai DC. A Lexicon of Medicinal Plants in India. Naya prakash publications Calcutta, India; 2001, p. 186-7.
8. MacLeod A, Pieris N. Volatile flavor components of Wood Apple (Feronia limonia) and a processed product. *J Agric Food Chem* 1981; 29: 49-53.
9. Wijeratne E, Bandara B, Gunatilaka A. Chemical constituents of three rutaceae species from Srilanka. *J Nat Prod* 1992; 55: 1262-9.
10. Mukhlesur M, Gray A. Antimicrobial constituents from the stem bark of Feronia limonia. *Phytochemistry* 2002; 59: 73-7.

11. Jain M, Trivedi A, Mishra S. TLC Determination of marmesin, a biologically active marker from *Feronia limonia* L. *Am J Plant Sci* 2010; 1: 12-6.
12. Jain M, Kapadia R, Jadeja RN, Thounaojam MC, Devkar RV, Mishra SH. Cytotoxicity evaluation and hepatoprotective potential of bioassay guided fractions from *Feronia limonia* Linn leaf. *Asian Pacific J Trop Biomed* 2011; 1: 443-7.
13. Jain M, Kapadia R, Jadeja RN, Thounaojam MC, Devkar RV, Mishra SH. Hepatoprotective activity of *Feronia limonia* root. *J Pharm Pharmacol* 2012. Doi: 10.1111/j.2042-7158.2012.01481.
14. Jadeja RN, Thounaojam MC, Dandekar DS, Devkar RV, Ramachandran AV. *Clerodendron glandulosum*. Coleb extract ameliorates high fat diet/fatty acid induced lipotoxicity in experimental models of non-alcoholic steatohepatitis. *Food Chem Toxicol* 2010; 48: 3424-31.
15. OECD. In: handbook, Good Laboratory Practice (GLP), quality practices for regulated non clinical research and development TDR PRD/GLP/01.2, 2001.
16. Jadeja RN, Thounaojam MC, Ansarullah, Jadav SV, Patel MD, Patel DK, Salunke SP, et al. Toxicological evaluation and hepatoprotective potential of *Clerodendron glandulosum* Coleb leaf extract. *Hum Exp Toxicol* 2011; 30: 63-70.
17. Kakkar P, Das B, Viswanathan P. A modified spectrophotometric assay of SOD. *Indian J Biochem Biophys* 1984; 21: 130-2.
18. Sinha K. Colorimetric assay of catalase. *Anal Biochem* 1972; 47: 389-94.
19. Beutler E, Duron O, Kelly B. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963; 61: 882-8.
20. Lowry O, Rosenbrough N, Farr A, Randall R. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.
21. Buege J, Aust S. Microsomal lipid peroxidation. *Methods Enzymol* 1978; 52: 302-10.
22. Roe J, Kuether C. The determination of ascorbic acid in whole blood and urine through 2-4 dinitrophenyl hydrazine derivative of dehydro ascorbic acid. *J Biol Chem* 1943; 147: 399-407.
23. Tshikalange T, Hussein A. Cytotoxicity activity of isolated compounds from *Elaeodendron transvaalense* ethanol extract. *J Med Plant Res* 2010; 4: 1695-7.
24. Noh JR, Gang GT, Kim YH, Yang KJ, Hwang JH, Lee HS, Oh WK, et al. Antioxidant effects of the chestnut (*Castanea crenata*) inner shell extract in t-BHP-treated HepG2 cells, and CCl₄ and high-fat diet-treated mice. *Food Chem Toxicol* 2010; 48: 3177-83.
25. Li L, Li W, Kim Y, Lee, Y. *Chlorella vulgaris* extract ameliorates carbon tetrachloride-induced acute hepatic injury in mice. *Exp Toxicol Pathol* 2011; Doi:10.1016/j.etp.2011.01.012.
26. Nitha B, Fijesh P, Janardhanan K. Hepatoprotective activity of cultured mycelium of Morel mushroom, *Morchella esculenta*. *Exp Toxicol Pathol* 2011; Doi:10.1016/j.etp.2011.06.007.
27. Sreelatha S, Padma PR, Umadevi M. Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats. *Food Chem Toxicol* 2009; 47: 702-8.
28. Ruiz-Torres A, Kürten I, Rosenstock M. The dynamics of collagen deposition in liver damaged by CCl₄. *Beitr Pathol* 1976; 158: 287-95.
29. Fu Y, Zheng S, Lin J, Ryerse J, Chen A. Curcumin protects the rat liver from CCl₄-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. *Mol Pharmacol* 2008; 73: 399-409.
30. D'Argenio G, Amoruso D, Mazzone G, Vitaglione P, Romano A, Ribocco M, D'Armiento MR, et al. Garlic extract prevents CCl₄-induced liver fibrosis in rats: The role of tissue transglutaminase. *Dig Liver Dis* 2010; 42: 571-7.
31. Palanivel M, Balasubramanian R, Kumar R, Einstein J, Kumar E, Kumar M, Kunchu K, et al. Hepatoprotective and Antioxidant Effect of *Pisonia aculeata* L. against CCl₄-Induced Hepatic Damage in Rats. *Sci Pharm* 2008; 76: 203-15.
32. Ahmed AF, Mahmoud MF, Ouf MA, El-Fathaah EA. Amino-guanidine potentiates the hepatoprotective effect of silymarin in CCl₄ treated rats. *Ann Hepatol* 2011; 10: 207-15.
33. Dikshit P, Tyagi MK, Shukla K, Sharma S, Gambhir JK, Shukla R. Hepatoprotective effect of stem of *Musa sapientum* Linn in rats intoxicated with carbon tetrachloride. *Ann Hepatol* 2011; 10: 333-9.