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EVALUATION OF ANTIOXIDANT AND HEPATOPROTECTIVE POTENTIAL OF *FERONIA LIMONIA* IN RATS

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ABSTRACT

Prophylactic and curative hepatoprotective property of suspension of *Feronia limonia* fruit was studied against PCM induced hepatic damage in albino rats. Pre and post treatment with SFL, before and after PCM treatment reduced the biochemical markers of hepatic damage like SGPT, SGOT, serum ALP, serum cholesterol, total and direct bilirubin and tissue GSH. Histopathological studies also revealed that pre and post treatment with SFL, before and after PCM treatment protected the liver from PCM induced liver damage. In addition an antioxidant study was carried out by *in vitro* lipid peroxidation. In the present study it has been found that prophylactic study showed better hepatoprotection than curative activity.

KEYWORDS: Antioxidant, *Feronia limonia*, hepatoprotection, paracetamol.

INTRODUCTION

Health is one of the commonly mentioned motivations behind food choices. The functional foods offer a new kind of health message by promising particular effects caused by particular food products. A food can be regarded as functional if it is satisfactorily confirmed to affect beneficially one or more target functions in the body, beyond enough nutritional effects. These effects have generally been well defined, such as lowering blood cholesterol levels or fortification of bones (Saher *et al.*, 2004). The health benefits of nutraceuticals may provide dietary essentials and factors that protect against the environment in which we survive and the potentially pathological events we internally created. Nutraceuticals have been proven to offer physiological benefits or to reduce the risk of persistent disease, or both, beyond their basic nutritional functions (Morganti, 2009; Serafini and Testa, 2009). Liver disorders are still a global health problem (Balamurugan *et al.*, 2008). Liver disorders, caused by various agents like alcohol consumption, environmental toxins and viruses, remain one of the major fears to public health. Treatment options for common liver disorders such as cirrhosis, fatty liver, and chronic hepatitis are tricky. The effectiveness of treatments such as interferon, colchicine, penicillamine, and corticosteroids are unpredictable and the incidences of side effects are more.

Though the modern medicinal system has grown phenomenally, the drug for treating hepatic disease is still a dream. Hence, people are looking at the traditional system of medicine for remedies of liver disorders (Bhat *et al.*, 1996; Qureshi *et al.*, 2007).

In this background *Feronia limonia* commonly known as Kaitha or Wood Apple belonging to family Rutaceae is selected. *Feronia limonia* is a moderate-sized tree with straight sharp strong spines, the fruits are edible. In Ayurveda the fruits are considered as sour, sweet, acrid, with flavour and taste; difficult to digest; refrigerant, aphrodisiac, alexipharmic; cures cough, dysentery, heart diseases, vomiting; removes biliousness, "vata", "tridosah", and blood impurities, fatigue, thirst, hiccough; good for throat, asthma, tumours, opthalmia, leucorrhoea, the juice put in the ear cures earache. According to Unani medicine the fruits are cardiotonic, tonic to the liver and the lungs, astringent and binding, diuretic, strengthening the gums; the juice is good for stomatitis, and sore throat; useful in biliousness; topically it relieves pain due to stings of wasps and other insects (Jadeja *et al.*, 2005; Dwivedi, 2000; Singh *et al.*, 2002). The phytochemical studies reveal that the fruit contains flavonoids, fruit acids, vitamins and mineral. The dried pulp contains 15% of citric acid (Saima *et al.*, 2000; MacLeoad and Pieris, 1981; Intekhab and Aslam, 2009). The present study was aimed to evaluate hepatoprotective effect of suspension of *Feronia limonia* using paracetamol induced liver injury in rats.

MATERIALS AND METHODS

Plant material

The fruits of wood apple purchased from the local market of Akola and the pulp was shade dried. The dried pulp was pulverized in a grinder and passed through mesh #80.

Preparation of suspension of Feronia limonia (SFL)
The sieved powder of the pulp was suspended in 2% gum acacia aqueous solution before administration.

Animals
Albino Wister rats (150-250 g) and mice (25-35 g) were housed under standard conditions of constant temperature and lighting (12 hours light/dark cycle). They had free access to standard pellet diet (Gold Mohur Lipton Indian Ltd.) and water *ad libitum*. The institutional animal ethical committee of A.U., College of Pharmaceutical Sciences, Andhra University, Visakhapatnam approved by CPCSEA with registration number 515/01/a/ CPCSEA approved the study.

In vitro antioxidant assay
Determination of lipid peroxidation inhibiting activity by Fe^{2+} /ascorbate system according to Ohkawa (Ohkawa *et al.*, 1979). Rat liver tissue weighing 10 g was homogenized with a polytron homogenizer in ice-cold Tris-HCl buffer to produce a 25% w/v homogenate. Then it was centrifuged at 4000 rpm for 10 min. An aliquot of supernatant 0.1 ml was mixed with 0.1 ml of the SFL of different concentrations, followed by addition of 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06 mM) and 0.1 ml of ammonium ferrous sulphate (0.16 mM) and were incubated for one hour at 37 °C. The reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20% acetic acid (pH 3.5). The total volume was then made up to 4 ml by adding distilled water and kept in an oil bath at 100 °C for 1 hour. After the mixture had been cooled, 1 ml of distilled water and 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substance (TBARS) was measured at 532 nm. A control was prepared using 0.1 ml of respective vehicle in the place of SFL/ ascorbic acid.

Acute toxicity study
According to Organization for Economic Co-operation and Development (OECD) drafted guideline No. 426. The study was conducted on albino mice of either sex weighing between 25-35 g. They were fasted over night and maintained with water *ad libitum*. The SFL was administered at a dose level of 2000 mg/kg body weight. No deaths observed after 24 hours and hence it is considered to be cut-off dose and $1/10^{th}$ and $1/20^{th}$ of this cut-off is taken for further in vivo study.

HEPATOPROTECTIVE ACTIVITY
Preventive study
Albino Wister rats of either sex were selected and divided into 5 groups each containing six rats. Paracetamol (PCM), powder of the selected plants material and silymarin were dissolved in 2% gum acacia suspension. The treatment protocol was planned to study the effect of SFL in preventive aspect of paracetamol induced hepatotoxicity (Shenoy *et al.*, 2002). The treatment protocol is summarized and given below.

Group I- Normal control, 2% w/v gum acacia suspension orally, 1 ml/kg once daily for 3 days
Group II- Paracetamol as toxicant 2 g/kg orally once daily for 3 days
Group III- SFL 200 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days
Group IV- SFL 400 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days
Group V- Silymarin 100 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days

On 0^{th} day (one day before the dosing) blood was collected by retroorbital puncture from all the animals. Serum was separated by centrifugation (3000 rpm for 15 min.) and subjected for estimation of biochemical parameters such as SGPT, SGOT, ALP, serum cholesterol and bilirubin. Then on the 4^{th} day (next day of last dosing) the animals were sacrificed, blood was collected and the livers were isolated and washed with fresh saline. Small piece of fresh liver is used for GSH estimation. Livers were stored in 10 % formalin for histopathological study.

Curative study
The treatment protocol was planned to study the effect of functional foods in curative aspect of paracetamol induced hepatotoxicity (Shenoy *et al.*, 2002). The treatment protocol is summarized and given below.
Group I- Normal control, 2% w/v gum acacia suspension orally, 1 ml/kg once daily for 10 days
Group II- Paracetamol as toxicant 2 g/kg orally once daily for 3 days followed by 1 ml/kg 2% gum acacia suspension from 4^{th} day to 10^{th} day
Group III- PCM 2 g/kg orally for 3 days followed SFL 200 mg/kg orally from 4^{th} day to 10^{th} day
Group IV- PCM 2 g/kg orally for 3 days followed SFL 400 mg/kg orally from 4^{th} day to 10^{th} day
Group V- PCM 2 g/kg orally for 3 days followed Silymarin 100 mg/kg orally from 4^{th} day to 10^{th} day

On 0^{th} day (one day before the dosing) blood was collected by retroorbital puncture from all the animals. Serum was separated by centrifugation (3000 rpm for 15 min.) and subjected for estimation of biochemical parameters such as, SGPT, SGOT, ALP, cholesterol and bilirubin, as described in chapter 3. Then on the 11^{th} day (next day of last dosing) the animals were sacrificed, blood was collected and the livers were isolated and washed with fresh saline. Livers were stored in 10 % formalin for histopathological study.

***In-vivo* antioxidant assay**
Tissue samples, after preventive treatment, were homogenized in ice cold Trichloroacetic acid (1 gm tissue plus 10 ml 10% TCA) in an ultra turrax tissue homogenizer. Briefly, after centrifugation at 3000 rpm

for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of Dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. Glutathione measurements were performed using a modification of the Ellman procedure (Aykae *et al.*, 1985).

Statistical analysis

Results were expressed as mean ± SEM, (n=6). Statistical analysis was performed with one way analysis of variance (1 way ANOVA) followed by Tukey test. P value less than 0.05 was considered to be statistically significant. *=P<0.05, **=P<0.01 and ***=P<0.001, when compared with toxicant group. 50% inhibition concentration for superoxide anion scavenging activity has been calculated by Microsoft Excel programme.

Histopathological observations

Liver tissue collected were used for the preparation of histopathological studies by using microtome and were suitably stained and observed under microscope for architectural changes seen during paracetamol challenge in SFL treated and control group.

RESULTS AND DISCUSSION

SFL and ascorbic acid at different concentrations (12.5-400 µg) inhibited the lipid peroxidation in a dose dependent manner. The amount needed for 50% inhibition of lipid peroxide was found to be 424.15µg and 179.66µg respectively (Table 1).

Table 1: *In vitro* lipid peroxidation of SFL

Treatments	Quantity (µg)						IC_{50} Value (µg)
	12.5	25	50	100	200	400	
Feronia limonia	0.123±0.01 (2.5)	0.125±0.01 (4.17)	0.128±0.02 (6.67)	0.134±0.02 (11.67)	0.156±0.01 (30.0)	0.174±0.01 (45.0)	424.15
Ascorbic acid	0.138±0.01 (15.0)	0.161±0.01 (34.17)	0.173±0.01 (44.17)	0.184±0.01 (53.33)	0.190±0.01 (58.33)	0.199±0.05 (65.83)	179.66
Control	0.12±0.01						

Values are the mean ± SEM, n=3; values in parenthesis indicate % inhibition

The dose of 2 g/kg body weight of paracetamol induced significant increase in SGPT, SGOT, serum ALP, serum cholesterol, total bilirubin and direct bilirubin levels when compared to positive control as well as when compared to day 0 value of the same group in paracetamol induced hepatotoxicity model. The percentage change in serum enzyme levels from 0^{th} day to 4^{th} day in prophylactic study and on 11^{th} day in curative study was also calculated. The % change of SGPT, SGOT, serum ALP, serum cholesterol, total bilirubin and direct bilirubin in prophylactic study was 292.87, 447.14, 300.5, 145.22, 281.34 and 63.07% in toxicant group. These % change has been decreased in a dose dependent manner by SFL (Table 2). The % change of SGPT, SGOT, serum ALP, serum cholesterol, total bilirubin and direct bilirubin in curative study, on 11^{th} day, was 258.63, 408.37, 225.0, 109.96, 237.21 and 133.33% in toxicant group. These % changes are more compared to prophylactic study (Table 3). Prophylactic treatment with SFL showed better results than curative treatment in paracetamol induced hepatotoxicity study.

Table 2: Influence of SFL on biochemical parameters in rats for PCM induced toxicity (Prophylactic study)

Treatment / Parameters	Group I 2% Gum acacia (1 ml/kg)	Group II PCM (2 g/kg)	Group III PCM+ SFL (2g/kg+200mg/kg)	Group IV PCM + SFL (2g/kg+400mg/kg)	Group V PCM+ Silymarin (2g/kg+100mg/kg)
SGPT (0^{th} Day)	36.10 ± 2.88	33.09 ± 0.99	32.22 ± 1.26	31.48 ± 1.4	30.95 ± 0.8
SGPT (4^{th} Day)	36.85 ± 2.22 (2.08)	130.0 ± 3.91 (292.87)	49.02 ± 5.68*** (52.14)	43.00 ± 4.90*** (36.59)	33.03 ± 3.30*** (6.72)
SGOT (0^{th} Day)	81.91 ± 4.47	82.30 ±1.83	82.12 ± 1.04	81.32 ± 1.98	80.58 ± 2.04
SGOT (4^{th} Day)	83.00 ± 3.46 (1.33)	450.3 ± 16.68 (447.14)	129.1 ± 11.07*** (57.21)	110.2 ± 4.82*** (35.51)	90.01 ± 4.74*** (11.7)
ALP (0^{th} Day)	182.4 ± 5.84	180.3 ± 4.28	181.5 ± 2.98	180.8 ± 4.14	182.3 ± 2.66
ALP (4^{th} Day)	184.2 ± 4.99 (0.99)	722.1 ± 14.56 (300.50)	301.2 ± 6.99*** (65.95)	255.2 ± 9.39*** (41.15)	201.2 ± 18.08*** (10.37)
Cholesterol (0^{th} Day)	100.8 ± 1.37	99.95 ± 1.76	101.1 ± 3.73	102.7 ± 2.41	101.5 ± 3.1
Cholesterol (4^{th} Day)	102.2 ± 5.72 (1.39)	245.1 ± 13.53 (145.22)	135.1 ± 11.51*** (33.63)	127.0 ± 8.02*** (23.66)	108.0 ± 5.33*** (6.4)
Total Bilirubin (0^{th} Day)	0.89 ±0.075	0.82 ± 0.04	0.91 ± 0.05	0.92 ± 0.18	0.92 ± 0.04
Total Bilirubin (4^{th} Day)	0.90 ± 0.05 (1.49)	3.13 ± 0.37 (281.34)	1.32 ± 0.08*** (44.51)	1.12 ± 0.12*** (21.96)	1.00 ± 0.04*** (8.91)
Direct Bilirubin (0^{th} Day)	0.23 ± 0.03	0.14 ± 0.02	0.17 ± 0.03	0.21 ± 0.04	0.16 ± 0.04
Direct Bilirubin (4^{th} Day)	0.23 ±0.03 (0.0)	0.23 ± 0.04 (63.07)	0.23 ± 0.04 (37.23)	0.26 ± 0.02 (22.24)	0.17 ± 0.02 (7.31)

*Values are the mean ± S.E.M. of six rats/treatment; *** **P<0.001, *P<0.01, P<0.05 compared to PCM treatment Group II)*
Values in parenthesis indicate % change on 4^{th} day.

Table 3: Influence of SFL on biochemical parameters in rats for PCM induced toxicity (Curative study)

Treatment / Parameters	Group I 2% Gum acacia (1 ml/kg)	Group II PCM (2 g/kg)	Group III PCM+ SFL (2g/kg+200mg/kg)	Group IV PCM + SFL (2g/kg+400mg/kg)	Group V PCM+ Silymarin (2g/kg+100mg/kg)
SGPT (0^{th} Day)	33.74 ± 1.95	33.21 ± 1.66	31.25 ± 1.14	31.44 ± 1.57	31.48 ± 1.40
SGPT (11^{th} Day)	34.60 ± 2.73 (2.55)	119.1 ± 8.76 (258.63)	50.07 ± 5.55*** (60.22)	44.99 ± 4.74*** (43.09)	35.37 ± 3.71*** (12.36)
SGOT (0^{th} Day)	83.18 ± 2.97	80.67 ± 2.85	81.46 ± 3.48	82.46 ± 1.81	81.89 ± 2.14
SGOT (11^{th} Day)	84.19 ± 3.79 (1.22)	410.1 ± 5.19 (408.37)	135.0 ± 6.86*** (65.73)	117.3 ± 3.16*** (42.25)	96.06 ± 4.29*** (17.30)
ALP (0^{th} Day)	181.9 ± 4.35	180.0 ± 3.46	181.2 ± 2.41	180.3 ± 4.42	182.6 ± 2.37
ALP (11^{th} Day)	184.2 ± 3.68 (1.26)	585.0 ± 35.53 (225.00)	315.0 ± 7.23*** (73.84)	270.1 ± 16.25*** (49.81)	213.9 ± 13.55*** (17.14)
Cholesterol (0^{th} Day)	99.17 ± 3.57	99.16 ± 2.90	99.00 ± 3.07	100.6 ± 2.25	99.13 ± 2.77
Cholesterol (11^{th} Day)	100.1 ± 2.58 (0.94)	208.2 ± 3.82 (109.96)	141.2 ± 14.9*** (42.63)	131.2 ± 8.81*** (30.42)	113.2 ± 4.83*** (14.19)
Total Bilirubin (0^{th} Day)	0.84 ± 0.02	0.86 ± 0.02	0.88 ± 0.02	0.89 ± 0.02	0.88 ± 0.03
Total Bilirubin (11^{th} Day)	0.852 ± 0.02 (1.19)	2.95 ± 0.08 (237.21)	1.40 ± 0.07*** (59.09)	1.22 ± 0.07*** (34.83)	1.02 ± 0.05*** (13.64)
Direct Bilirubin (0^{th} Day)	0.16 ± 0.02	0.18 ± 0.03	0.18 ± 0.03	0.21 ± 0.03	0.25 ± 0.03
Direct Bilirubin (11^{th} Day)	0.16 ± 0.01 (0.00)	0.42 ± 0.05 (133.33)	0.26 ± 0.02* (44.44)	0.27 ± 0.02 (28.57)	0.28 ± 0.04 (12.00)

*Values are the mean ± S.E.M. of six rats/treatment; ***P<0.001, **P<0.01, *P<0.05 compared to PCM treatment (Group II)*
Values in parenthesis indicate % change on 11^{th} day.

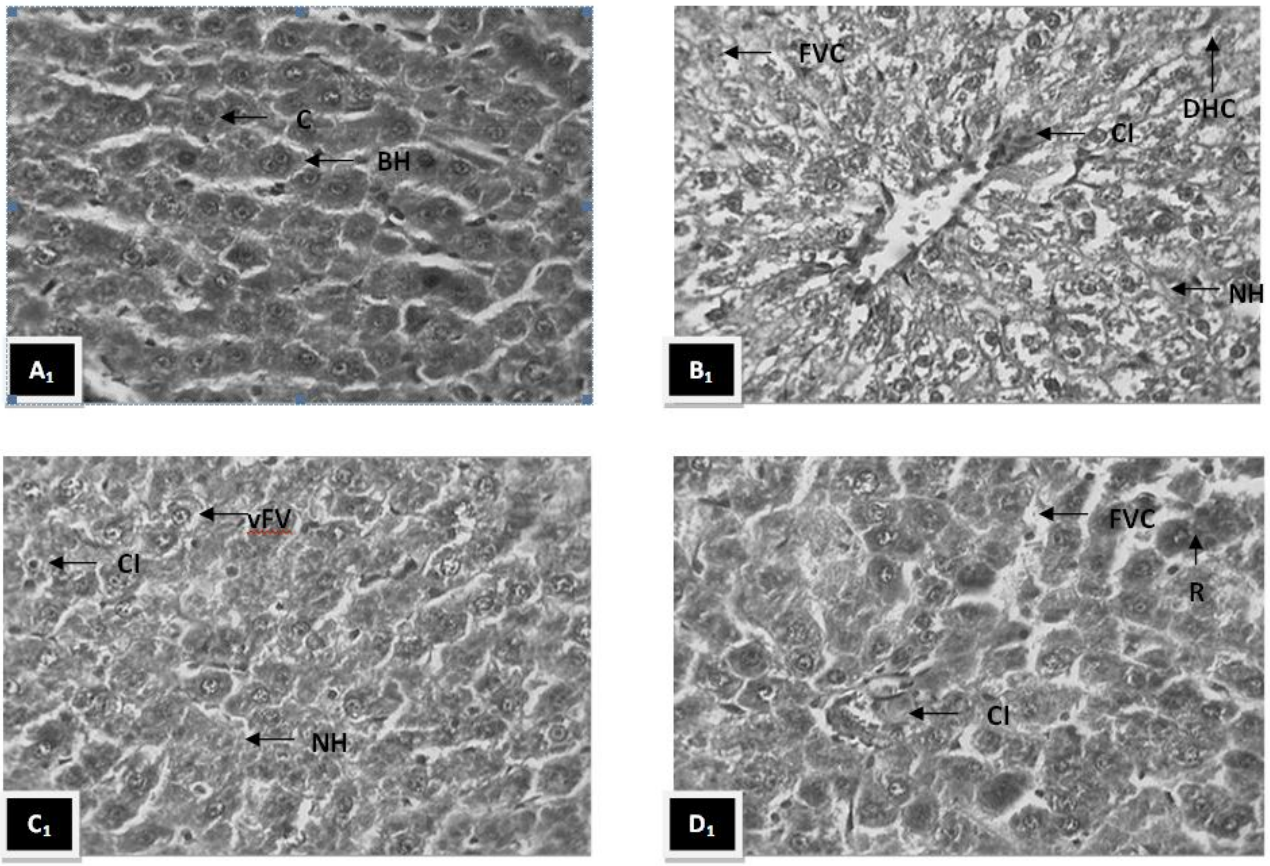
Three day's treatment of 2 g/kg PCM drastically decreased tissue glutathione levels which are elevated by SFL but the elevation of glutathione is far better by silymarin.

Table 4: Influence of the selected functional foods on GSH levels in paracetamol induced hepatotoxicity

Gr.	Treatment	Absorbance (Mean ± S.E.M.)	% Increase
1.	2% Gum acacia (1 ml/kg; p.o.)	0.931 ± 0.0573	--
2.	PCM (2 g/kg; p.o.)	0.326 ± 0.0731	--
3.	PCM + *Feronia limonia* (2 g/kg; p.o. + 200 mg/kg; p.o)	0.401 ± 0.0271**	23.00
4.	PCM + *Feronia limonia* (2 g/kg; p.o. + 400 mg/kg; p.o)	0.437 ± 0.0341***	34.09
5.	PCM + Silymarin (2 g/kg; p.o. + 100 mg/kg; p.o)	0.586 ± 0.0341***	79.75

Values are the mean ± S.E.M. of six rats/treatment
****P<0.001, **P<0.01, *P<0.05 compared to PCM treatment*

In case of paracetamol treated group (toxicant control) derangement of cords, fatty and vacuolar degeneration, necrosis and cellular infiltration have been observed. Whereas lower and higher dose of SFL showed less fatty and vacuolar degeneration, necrosis, cellular infiltration and derangement of cord have been observed. Mild regeneration is also observed with higher dose. 100 mg/kg of silymarin showed very less derangement of cords. Very good regeneration is also observed with normal hepatic features. Similar to biochemical studies, prophylactic study showed better hepatic architecture than in curative study.



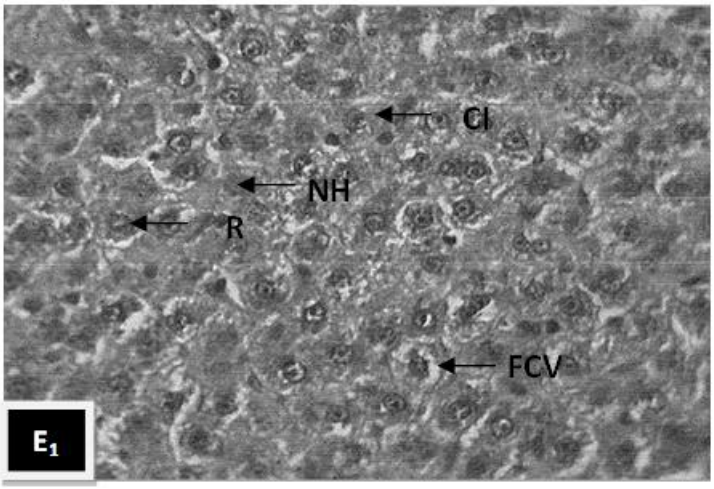
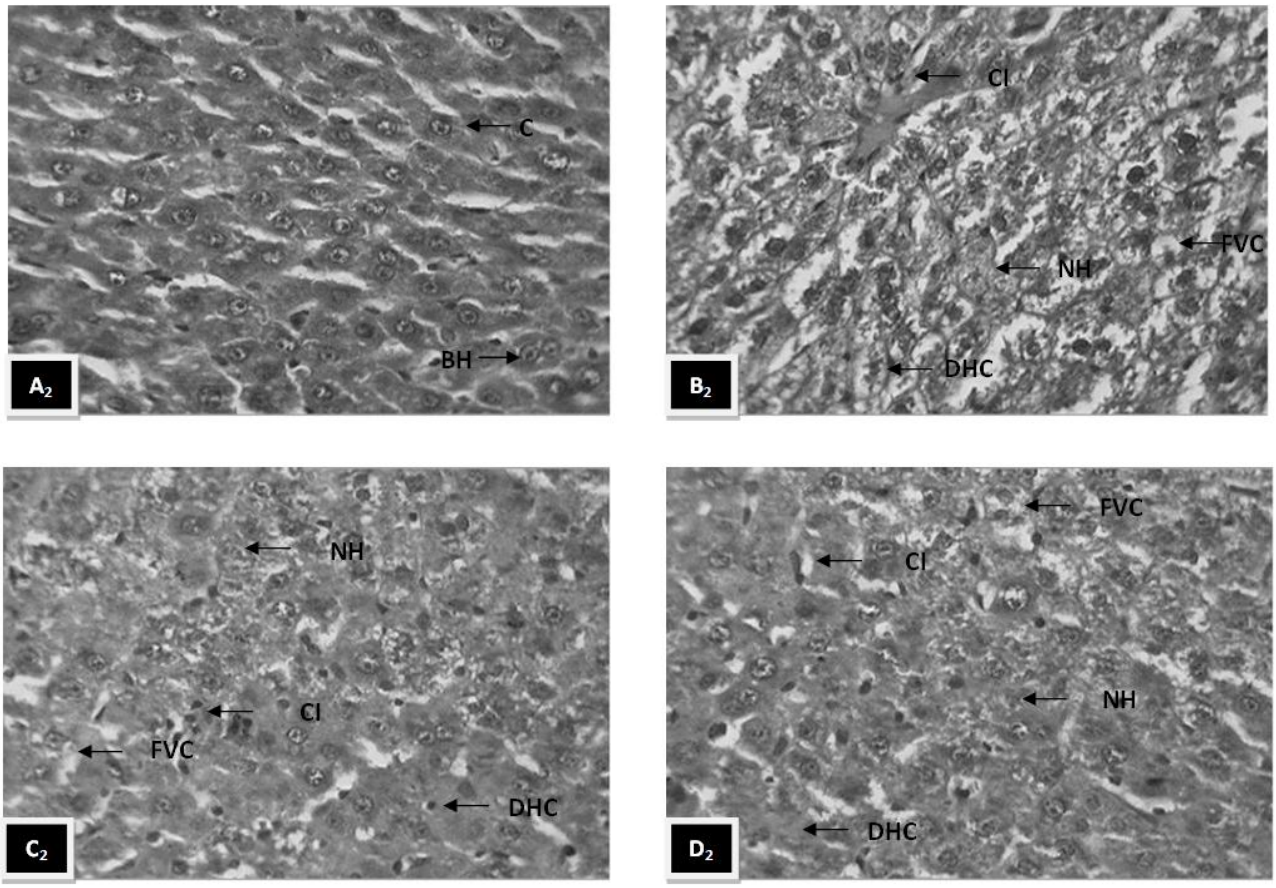


Fig. 1: Histopathology of prophylactic study (A_1) Normal control group show normal hepatic globular structure without any abnormality: (B_1) Toxicant group showed up to 75% derangement of cords, fatty and vacuolar degeneration, necrosis and cellular infiltration: (C_1) 200 mg/kg of *Feronia limonia* showed less than 50% fatty and vacuolar degeneration and necrosis whereas less than 25% cellular infiltration and derangement of cord have been observed. Mild regeneration is also observed: (D_1) 400 mg/kg of *Feronia limonia* less than 50% fatty and vacuolar degeneration whereas less than 25% necrosis, cellular infiltration and derangement of cord have been observed. Moderate regeneration is also observed: (E_1) 100 mg/kg of silymarin showed less than 25% derangement of cords. Very good regeneration is also observed with normal hepatic features X400 (BH: Binucleated Hepatocytes; C: Cytoplasm; CI: Cellular Infiltration; DHC: Derangement of Hepatic Cords; FVC: Fatty and Vacuolar Changes; NH: Necrosis of Hepatocytes; R: Regeneration)



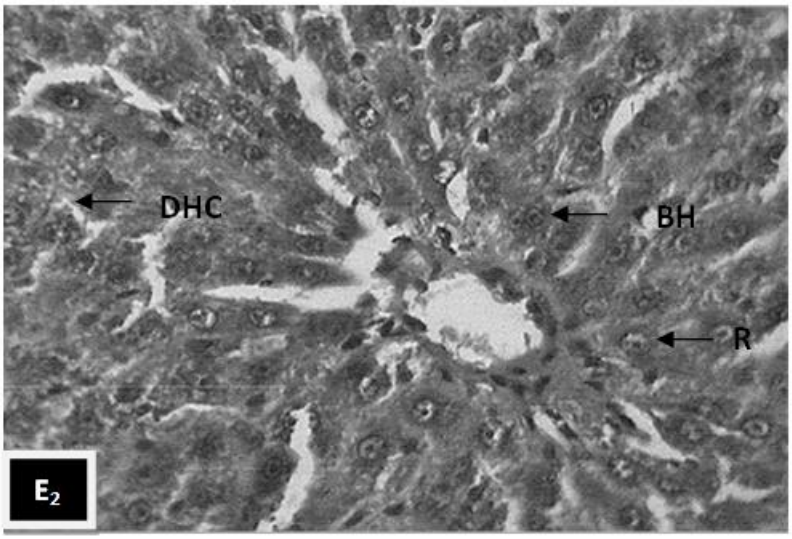


Fig. 2: Histopathology of curative study (A_2) Normal control group show normal hepatic globular structure without any abnormality: (B_2) Toxicant group showed more than 75% derangement of cords, fatty and vacuolar degeneration, necrosis and cellular infiltration; (C_2) 200 mg/kg of *Feronia limonia* showed less than 50% fatty and vacuolar degeneration, necrosis and cellular infiltration whereas less than 25% derangement of cord have been observed: (D_2) 400 mg/kg of *Feronia limonia* less than 50% fatty and vacuolar degeneration and necrosis whereas less than 25% cellular infiltration and derangement of cord have been observed. Mild regeneration is also observed: (E_2) 100 mg/kg of silymarin showed less than 25% derangement of cords. Very good regeneration is also observed with normal hepatic features X400 (BH: Binucleated Hepatocytes; C: Cytoplasm; CI: Cellular Infiltration; DHC: Derangement of Hepatic Cords; FVC: Fatty and Vacuolar Changes; NH: Necrosis of Hepatocytes; R: Regeneration)

There are potent native herbal medicines available for liver disorders in various parts of the world and much of them are not yet scientifically validated and if done may lead to the development of cost effective drugs. In this background our present study was planned to evaluate the *Feronia limonia* fruits for hepatoprotective activities against paracetamol induced hepatic damage in animal models.

Drug induced injury is the second main cause of acute liver failure and predominates in much of the developed world (Bernal *et al.*, 2010). PCM induced hepatotoxicity is the characteristic form of acute liver failure. The clinical course is often rapidly progressive multi-organ failure, with a greater severity of illness than that seen in liver failure from other causes (Bernal *et al.*, 2009). More than 56,000 emergency visits and nearly 500 deaths in the United States each year result from paracetamol toxicity, owing to either deliberate or unintentional overdoses (Timothy *et al.*, 2006).

PCM is normally eliminated mainly as sulfate and glucoronide. Only 5% of the PCM is converted into N-acetyl-p-benzoquineimine. However, upon administration of toxic doses of PCM the sulfation and glucoronidation routes become flooded and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquineimine (NAPQI) by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently binds to macromolecules of cellular membrane and increases the lipid peroxidation follow-on in the tissue damage. Higher dose of PCM and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently, leading to increased lipid peroxidation and liver damage (Setty *et al.*, 2007). An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disturbances caused in the transport functions of hepatocytes. When liver cell plasma is damaged, a variety of enzymes like SGOT, SGPT, ALP, and Bilirubin located normally in cytosol are released into the blood, thereby causing increased enzyme levels in the serum. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Ghosh and Sil, 2009; Sadasivan *et al.*, 2006). The abnormal high level of serum biomarker enzymes and bilirubin observed in this study are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Oral administration of SFL exhibited a significant reduction in paracetamol induced levels of serum GOT, GPT, ALP and bilirubin value remarkably to the normal group that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretary mechanism of the hepatic cell. It has been indicated that micro viscosity of a membrane increases markedly with increase in cholesterol level in plasma. PCM induced toxicity in rats may have altered membrane structure and function as

suggested by the increases in cholesterol. This result is an indication of membrane rigidity caused by PCM. Alteration of bio-membrane lipid profile disturbs its fluidity, permeability, activity of associated enzymes and transport system (Ashokkumar *et al.,* 2008). In this study it has been observed that cholesterol levels have been increased due to paracetamol treatment. However, treatment of rats with SFL inhibited the alteration of lipid membranes and fluidity hence lowered cholesterol level in plasma. The higher dose showed better hepatoprotection compared to lower dose of SFL. The histopathological study further supports the findings of biochemical parameters (Fig 1 and 2).

Lipid peroxidation has been postulated as being a destructive process in liver injury caused by PCM administration (Muriel *et al.*, 1992). In our study, the in vitro lipid peroxidation of SFL showed percentage reduction comparable with standard ascorbic acid (Table 1). The IC_{50} value for the SFL and standard ascorbic acid was found to be 424.5 µg and 179.66 µg respectively. Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase (GPx) (Ashokkumar *et al.*, 2008). In our present study the lowered level of GSH due to PCM treatment was increased by SFL in a dose dependent manner (Table 4).

Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P_{450}. Induction of cytochrome P_{450} or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity (Ashokkumar *et al.,* 2008). Therefore the antihepatotoxic activity of the drug may be due to: inhibition of cytochrome P_{450}, promotion of glucuronidation, stimulation of hepatic regeneration or activation of the functions of the reticulo endothelial systems.

CONCLUSION

Today we realized that diets rich in bioactive phytochemicals reduce the risk of degenerative disorders such as cancer, diabetes, cardiovascular and oxidative dysfunction. Foods containing these phytochemicals not only can provide our diet with certain antioxidant vitamins like Vitamin C, Vitamin E and pro Vitamin A but also a complex mixture of other natural substances with antioxidant capacity (Muriel *et al.,* 1992). In Indian cooking system we generally over cook our foods and destroyed their essential nutrients and also important antioxidants. It has been proved also that heat treatments affect the antioxidant activity of vegetables and in many cases has been observed lower antioxidant capacity in processed samples versus raw vegetables. Nutrient antioxidants may act jointly to reduce reactive oxygen species level more effectively than single dietary antioxidants, because they can function as synergists (Natarajan *et al.*, 2006; Amin *et al.*, 2006; Podsedek, 2007; Suresh *et al.*, 2007). Hence consuming raw vegetables, fruits and nuts are more beneficial than cooking them harshly.

In conclusion, the SFL possess antioxidant and hepatoprotective activity. In the present study it has been found that prophylactic study showed better hepatoprotection than curative activity which may further support the antioxidant mechanism behind hepatoprotection, in prophylactic activity as lipid peroxidation and protection of GSH may be the reason behind better hepatoprotection in prophylactic study.

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