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# FLACOURTIA INDICA (BURM.F.) PREVENTS $CCL_4$ INDUCED RAT LIVER DAMAGE BY AUGMENTING ANTIOXIDANT ENZYME ACTIVITY

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## ABSTRACT

**Aim of the study:** *Flacourtia indica* (Burm.f.) (Family-Flacourtiaceae) is a nontoxic edible herb, widely used in Indian folklore medicine to treat different types of liver ailments. In the present study we have demonstrated that methanol extract of *F. indica* fruit could prevent $CCl_4$ induced rat liver damage. **Materials and methods:** $CCl_4$ at the dose of 1.5 ml/kg body wt. was orally administered to experimental rats and liver function enzymes such as AST, ALT, ALP, lipid peroxidation and various antioxidant enzyme activities were measured. Effects of *F. indica* fruit extract at a dose of 250mg/kg body weight and 500mg/kg body weight on $CCl_4$ treated rats were checked. **Results:** $CCl_4$ produced significant increase in liver marker enzyme activities and reduction of antioxidant enzyme activities. Pre-treatment with methanolic extract of *F. indica* fruit in both doses 250 mg/kg and 500 mg/kg reversed the liver damage due to $CCl_4$ administration. It decreased the activities of liver enzymes and augmented antioxidant enzymes. Histopathological evaluation of liver also revealed that *F. indica* fruit extract reduced the incidence of $CCl_4$ induced liver lesions. The results obtained were compared with standard hepatoprotective drug silymarin (50 mg/kg) and found significant hepatoprotective activity similar to silymarin. **Conclusion:** *F. indica* fruit extract therefore shows a promise in therapeutic use in $CCl_4$ induced liver dysfunction.

**KEY WARDS:** Flacourtia indica, $CCl_4$, hepatoprotective, antioxidant activity.

## INTRODUCTION

Liver, one of the most imperative organs in body, provides protection against harmful foreign substances by detoxifying and eliminating them. Because of this vital role played by liver makes it susceptible to first and persistent attack by offending by alien materials culminating in liver dysfunction. Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe.[1] Alcohol consumption is the principal causative factors for the liver diseases in developed countries, where as in developing countries most frequent causes are environmental toxins, parasitic diseases, hepatitis B and C viruses, drugs like antibiotics, chemotherapeutic agents, overdose of paracetamol, carbon tetrachloride ($CCl_4$), thioacetamide (TAA) etc.[2,3] Among them Carbon tetrachloride is a common industrial solvent which is well known for its hepatotoxicity.[4-6] Occupational exposure to carbon tetrachloride may occur in the chemical industry, in laboratories, and during degreasing operations.[7,8] Through the investigation of acute $CCl_4$-induced liver damage in animal models, it is now generally accepted that $CCl_4$ toxicity resulted from free radical generation by cytochrome P450 system radicals that can bind covalently to cellular macromolecules forming nucleic acid, protein and lipid adducts in liver microsomes and consequently causes lipid peroxidation of membranes that leads to liver injury.[9-12] Overall, $CCl_4$ treatment can result in centrilobular steatosis, inflammation, apoptosis and necrosis.[13,14] If the damage exceeds the repair capacity of the liver, the liver will progress to fibrosis and cirrhosis.[15]

Since time immemorial, mankind has made the use of plants in the treatment of various ailments. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practice as well as traditional system of medicine in India.[16-18] Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness.[19] A number of studies have shown that the plant extracts having antioxidant activity protect against $CCl_4$ hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity.[20,21] Despite the significant popularity of herbal remedies for liver disorder, they are still unacceptable due to lack of scientific evidences.

*Flacourtia indica* (Burm.f.) (Family-Flacourtiaceae), commonly known as 'boichi', 'indian plum' is a herbal medicinal plant native to Africa and widely distributed in

various parts of India and Bangladesh.[22] This is a busy shrub of small tree with strong spiny, erect, branches, growing upto maximum height of 15 mt. Each part (leaves, bark, stem, fruits, root and even whole plant) of the Flacourtia indica has demonstrated several pharmacological activities including Anti-Inflammatory, Antimicrobial, Antioxidant, Hepatoprotective, Antimalarial, Anti-Diabetic, Anti asthmatic and Antibacterial activity. [23,24] Fruits are used as appetizing and digestive, diuretic, in jaundice and enlarged spleen. Barks are used for the treatment of intermittent fever. Roots are used in nephritic colic and gum is used in cholera.[22,25,26] Phytochemical investigation on this plant showed the presence of flavonoids, poly phenols like β-sitosterol, β-sitosterol-β-D-glucopyranoside, ramontoside, butyrolactone lignan disaccharideand flacourtin, coumarin such as scoparone and aesculetin.[27-29]

In the view of scientific report, fruits of Flacourtia indica was evaluated against $CCl_4$ induced hepatic damage in rats with the aim of developing a natural hepatoprotective drug.

## MATERIALS AND METHODS

### Chemicals

$CCl_4$ was obtained from Merck, Germany and silymarin was purchased from Sigma chemicals, USA. 1-Chloro2,4-dinitro benzoic acid (CDNB) and reduced glutathione (GSH) were supplied by Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Thiobarbituric acid and 5,5'-dithio-bis2-nitrobenzoic acid (DTNB) were purchased from E-Merck, India. Kits for determination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatise (ALP), total bilirubin, total protein and total cholesterol levels were purchased from Span diagnostics Ltd, Gujarat, India. All other chemicals used were of analytical grade.

### Collection of plant material and preparation of plant extract

The fresh mature fruits of *F. indica* were collected from Kanyakumari District, Tamilnadu, India and authenticated by taxonomist Prof. M. Venkaiah, Department of Botany, Andhra University, India. A voucher specimen was deposited in the Herbarium of pharmacognosy department of Andhra University, India for reference (AUPH15). The shade dried 1 kg fruits were made into coarse powder and subjected to extraction with 70% v/v methanol using a soxhlet extractor for 48 hrs. The total methanol extract was filtered and concentrated under reduced pressure at a room temperature not exceeding 40$^{o}$C to get the extract (110 g). This extract was stored in airtight containers in dark at – 4$^{o}$C until used for experiments.

### Phytochemical screening

The methanolic extract obtained from fruits of *F. indica* was subjected to preliminary phytochemical screening to identify the chemical constituents. The methods of analysis were performed according to the protocol suggested by Trease and Evans, 1989; Harbone and baxter, 1993 and Kandelwal, 2004.[30-32]

### Determination of Acute Oral Toxicity ($LD_{50}$)

Acute oral toxicity of methanol extract of *F. indica* fruits was determined as per OECD (Organisation for Economic Co-operation and Development) guideline 420.[33] Swiss albino mice of either sex weighing between 18-25 g were randomly selected, identified individually and kept in their cages for at least 5 days prior to dosing and allowed them for acclimatization under standard laboratory conditions. The dose levels of 5 mg, 50 mg, 100 mg, 200 mg, 500 mg, 1000 mg and 2000 mg/kg/body weight were selected. The animals were fasted overnight with water ad libitum prior to the administration of drug dose. The body weight of rat was noted before and after drug administration. Single dose of methanol extract suspended in 2% w/v acacia was administered orally and observed for toxic symptoms, such as behavioural changes, loco-motion, convulsion and mortality continuously for first 4 hrs, then overnight and finally for a time period of 72 hrs (short term toxicity). Based on short-term profile of drug, the dose of next animals was determined as per OECD guideline 425. All the animals were also observed for long term toxicity (14 days).

### Animals

Adult male albino Sprague-Dawley rats weighting 180 g to 225 g were randomly selected and maintained under standard laboratory conditions at an ambient temperature of 23±2$^{o}$C having 50±5% relative humidity with 12-h light and dark cycle. Animals were housed in polypropylene cages (3 animals in each) and fed standard pellet diet and allowed free access to water given ad libitum. The principles of laboratory animal care were followed throughout the experimental schedule and before performing the experiment the ethical clearance was obtained from institutional animal ethics committee (IEAC).

### Experimental design for hepatoprotective effect against CCl4-induced hepatotoxicity in rats

Animals were divided into five groups of six rats each. Group I and II served as normal and toxic control, and received only the vehicle (5% gum acacia; 1 ml/kg; p.o). Group III and IV received the methanolic extract of *F. indica* at an oral dose of 250 and 500 mg/kg respectively, as a fine suspension of 5% aqueous gum acacia. Group V animals were treated with standard silymarin at an oral dose of 100 mg/kg. The treatment was continued for 7 days, once daily. On the day of 7 for groups II-V, 30 min post-dose of extract administration animals received $CCl_4$ at the dose of 1.5 ml/kg (1:1 v/v of $CCl_4$ in olive oil) orally.[34,35] The animals were sacrificed after 36 h after administration of acute dose of $CCl_4$. Blood sample was withdrawn by cardiac puncture and serum was collected by clot retraction. Simultaneously livers were isolated and perfused with normal saline (0.9% NaCl) to

remove blood clot and rewashed thoroughly with ice cold phosphate buffer (50 mM, pH 7.4) with saline (PBS). 10% homogenates of liver tissue was prepared in PBS using homogeniser and then subjected to differential centrifugation to separate cytosolic and microsomal fractions according to methodology of Bock *et al.* (1979).[36] Protein estimation of the sample was done by using the method of Lowry *et al.* (1951).[37]

**Liver function assays**

Blood samples were drawn from ether anaesthetised rats by cardiac puncture and serum was collected by clot retraction. Serum AST, ALT, ALP, total bilirubin, total protein and total cholesterol levels were measured by using kits from Span Diagnostics Ltd., India, following the manufacturer's protocol.

**Estimation of TBARS**

The level of lipid peroxidation (LPO), as measured by TBARS, was determined according to the method of Buege and Aust (1978).[38] Briefly, 1 mi microsomal fraction (containing 1mg/ml protein) was mixed with 2 ml of the TBA-TCA-HCl reagent and vortexed thoroughly. After heating for 15 min in a boiling water bath, the samples were allowed to cool and den centrifuged at 1000×g for 10 min to remove the flocculants. The supernatant was checked for TBARS content as a measure of lipid peroxidation.

**Estimation of protein carbonyl content**

Protein carbonyl content was determined according to Levine *et al.* (1990),[39] using 0.8 ml of the cell free homogenate (10% homogenate centrifuged at 500×g for 10 min) in 50 nM sodium phosphate buffer, pH 7.4. The protein was suspended with 0.5 ml of 2, 4 dinitro phenylhydrazine and after washing in ethanol-ethyl acetate mixture (1:1), it was dissolved with 0.6 ml of guanidine-HCl and its absorbance was measured at 362 nm against blank. Protein carbonyl content was determined from the extinction coefficient at 362 nm ($\varepsilon$=22,000 $M^{-1}cm^{-1}$).

**Estimation of superoxide dismutase activity (SOD)**

Superoxide dismutase activity was assayed by its ability to inhibit the auto oxidation of hematoxylin into hematin following the method of Martin *et al.* (1987).[40] Liver homogenate was mixed with equal volume of 3:5 mixture of chloroform: ethanol and was then vortexed vigorously and centrifuged at 10000×g for 15 min. 10 µl of the upper ehanolic layer was added to 990 µl of 15 µM hematoxylin solution prepared in 50 mM phosphate buffer (pH 7.5). The decrease in absorbance was followed for 1 min at 556 nm. The results were expressed as unit SOD/mg protein.

**Estimation of catalase activity**

Catalase activity was assayed by method of Aebi (1984),[41] as modified by Kawamura (1999).[42] 20 µl of the cytosolic fraction was added to 980 µl of assay buffer containing 50mM Tris-HCl (pH 8.0), 9mM $H_2O_2$ and 0.25 mM EDTA to constitute the assay volume of 1 ml. The decrease in absorbance of that assay mixture was recorded at 240 nm for 1 min. The results were expressed as unit catalase/mg protein.

**Estimation of Glutathione (GSH)**

Glutathione was estimated by the method described by Senlac and Lindsay (1968).[43] Protein free supernatant was obtained by additional of an equal volume of 10% TCA to the tissue homogenate and centrifuged at 10,000 rpm for 10 min at 4°C. To 0.5ml of supernatant , 2.5 ml of 5,5'-dithio-bis2-nitrobenzoic acid (DTNB) prepared in 50mM phosphate buffer, pH 7.6 was added and vortexed. The absorbance was recorded spectrophotometrically at 405nm.

**Estimation of glutathione reductase (GR) activity**

Glutathione reductase (GR) activity was assayed accordingly to the method of Calberg and Mannervik (1985).[44] Activity was determined in the cytosolic fraction of liver by using 0.1 mM NADPH, 1 mM oxidised glutathione and 100 µl of the sample aliquots in a final assay volume of 1 ml. The disappearance of NADPH was measured at 340 nm for 1 min. The results were expressed as nmol NADPH oxidized/min (mg protein).

**Estimation of glutathione -S-transferase activity (GST)**

Glutathione-S-transferase activity was assayed in the liver cytosolic fractions by following the method described by Habig *et al.* (1974).[45] 1-Chloro-2, 4-dinitrobenzene (CDNB) was used as substrate in the presence of excess GSH. The rate of CDNB conjugation was determined spectrophotometrically at 340 nm for 2 min. The results were expressed as nmol/ min (mg protein).

**Histopathological studies**

Rat liver from each treatment group was excised and immediately fixed on Bouin's solution. After fixation the tissues were embedded in paraffin (52-54°C) to have 5 µm serial sections. The staining was done by routine hematoxylin-eosin (H&E) technique. The slides were examined under light microscope having photomicrographic attachment.

## RESULTS

**Phytochemical Screening**

The preliminary phytochemical screening of methanol extract of *F. indica* fruits showed that it contained alkaloids, flavonoids, coumarin glycosides, steroids, terpenoids, tannins and phenolic compounds, carbohydrates, proteins, amino acids. The result of preliminary phytochemical screening is complied in Table 1.

**Table 1: Phytochemical constituents present in methanolic extract of *F. indica* fruits**

| Phytoconstituents | Qualitative abundance |
|---|---|
| Alkaloids | ++ |
| Flavonoids | + |
| Cardiac Glycosides | -- |
| Coumarin Glycosides | +++ |
| Sterols | ++ |
| Saponins | + |
| Tannin & Phenolic compounds | +++ |
| Terpenoids | ++ |
| Caratenoids | -- |
| Lignans | ++ |
| Carbohydrates | ++ |
| Proteins & Amino acids | + |
| Volatile oil | -- |
| Acidic compounds | -- |

**Legend**
Levels of phytoconstituents were qualitatively determined based on chemical groups and thin layer chromatography on the following scale, -- absent, + present at low level, ++ present at moderate level, +++ present at high level.

**Acute toxicity ($LD_{50}$) studies**
Acute toxicity studies were carried out according to fixed dose method of OECD guideline no. 420. The oral administration of methanolic extract of *F. indica* fruits caused neither any behaviour changes nor mortality up to 2000 mg/kg. So the $LD_{50}$ of *F. indica* extract was found to be more than 2000 mg/kg.

**Liver function marker in serum**
A significant increase ($P<0.05$) in serum SGPT, SGOT, ALP, Total bilirubin and Cholesterol levels was observed in animals treated with $CCl_4$ as compared to normal. Pre-treatment with *F. indica* extract (250 mg/kg and 500 mg/kg p.o.) for 7 days decreases the above parameters significantly ($P<0.05$) as compared to $CCl_4$ treated group. Silymarin pre-treatment produced significant decrease ($p<0.05$) in the above parameter when compared to CCl4 treated group Total protein level which was significantly ($P<0.05$) declined in CCl4 treated group, restore their original value in case of co-administration with *F. indica* extract in both doses when compared with control rats (Table 2).

**Table 2: Effect of *F. indica* fruit extract on liver function marker enzymes and other biochemical parameters in CCl4 induced hepatotoxicity in rats**

| Groups | AST (IU/l) | ALT (IU/l) | ALP (IU/l) | Total Bilirubin (mg/dl) | Total Protein (g/dl) | Total Cholesterol (mg/dl) |
|---|---|---|---|---|---|---|
| Control **(I)** | 85.56 ± 3.14 | 70.63 ± 2.25 | 172.25 ± 1.28 | 0.37 ± 1.02 | 4.27 ± 0.34 | 50.67 ± 4.17 |
| $CCl_4$ **(II)** | 220.39 ± 5.68* | 159.18 ± 1.85* | 340.16 ± 4.79 * | 0.84 ± 0.70 * | 2.36 ± 0.72 * | 87.61 ± 1.95* |
| $CCl_4$ + *F. indica* fruit extract (250 mg/kg) **(III)** | 123.27 ± 4.37** | 98.67 ± 3.06** | 202.13 ± 5.44** | 0.63 ± 0.14** | 3.18 ± 0.42** | 65.71 ± 2.56** |
| $CCl_4$ + *F. indica* fruit extract (500 mg/kg) **(IV)** | 105.71 ± 2.21** | 80.15 ± 4.79** | 170.74 ± 3.62** | 0.47 ± 1.08** | 3.95 ± 0.65** | 60.81 ± 3.34** |
| $CCl_4$ + Silymarin (50 mg/kg) **(V)** | 96.03 ± 5.82** | 74.76 ± 2.58** | 181.32 ± 5.19** | 0.41 ± .69** | 4.45± 0.32** | 53.81 ± 2.58** |

**Legend**
Values are expressed as mean ± S.D. for six rats in each group, *P < 0.001 significantly different from control group (Gr. I), **P < 0.001 significantly different from the group treated with $CCl_4$ (Gr. II); AST denotes aspartate amino transferase, ALT denotes alanine amino transferase, ALP denotes alkaline phosphatase.

**Oxidative stress markers in liver tissue**
Administration of $CCl_4$ in rat produced oxidative stress on liver which was identified by significant ($P<0.05$) increase in lipid peroxidation, as determined by TBARS and protein carbonyl contents in CCl4 treated group in comparison to control. The deleterious effects of $CCl_4$ were waived by different doses of *F. indica* extract (Table 3).

**Table 3: Effect of *F. indica* fruit extract on the level of lipid peroxidation in $CCl_4$ induced hepatotoxicity in rats**

| Groups | TBARS (nmol/mg protein) | Protein carbonyl (nmol/mg protein) |
|---|---|---|
| Control **(I)** | 4.73 ± 1.02 | 5.73 ± 0.61 |
| $CCl_4$ **(II)** | 8.48 ± 0.65* | 11.42 ± 0.93* |
| $CCl_4$ + *F. indica* fruit extract (250 mg/kg) **(III)** | 6.17 ± 0.36** | 6.51 ± 0.42** |
| $CCl_4$ + *F. indica* fruit extract (500 mg/kg) **(IV)** | 5.51 ± 1.74** | 6.18 ± 0.62** |
| $CCl_4$ + Silymarin (50 mg/kg) **(V)** | 5.12 ± 0.53** | 5.89 ± 0.66** |

**Legend**
Values are expressed as mean ± S.D. for six rats in each group, *P < 0.001 significantly different from control group (Gr. I), **P < 0.001 significantly different from the group treated with $CCl_4$ (Gr. II.); TBARS denotes Thiobarbituric acid reactive substances.

As $CCl_4$ treatment powerfully inhibited the activities of antioxidant enzymes such as SOD, catalase, glutathione reductase and glutathione-S-transferase reconfirming that oxidative stress is existing in liver tissue due to $CCl_4$ administration to rats. The level of GSH in $CCl_4$ treated group was nearly half of the control group. The reduction of antioxidant enzyme activities was prevented significantly (P<0.05) by both doses of *F. indica* extract. However 500 mg/kg dose was found to be more effective in preventing the oxidative stress-induced liver damage due to administration of $CCl_4$ and these values are comparable with those values in silymarin treated group (Table 4).

**Table 4: Effect of *F. indica* fruit extract on antioxidant enzyme activities in $CCl_4$ induced hepatotoxicity in rats**

| Groups | SOD (Unit/mg protein) | Catalase (Unit/mg protein) | GSH (µg/mg protein) | GR (nmol/mg protein) | GST (nmol/mg protein) |
|---|---|---|---|---|---|
| Control **(I)** | 206.4 ± 10.3 | 165.7 ± 5.6 | 7.23 ± 0.54 | 82.5 ± 3.5 | 150.6 ± 6.1 |
| $CCl_4$ **(II)** | 88.7 ± 7.5* | 94.3 ± 2.6* | 3.15 ± 0.76* | 48.2 ± 5.7* | 72.5 ± 8.5* |
| $CCl_4$ + *F. indica* fruit extract (250 mg/kg) **(III)** | 153.6 ± 9.1** | 119.5 ± 6.3** | 5.89 ± 0.42** | 63.8 ± 4.8** | 121.6 ± 3.4** |
| $CCl_4$ + *F. indica* fruit extract (500 mg/kg) **(IV)** | 171.4 ± 7.6** | 140.8 ± 3.8** | 6.32 ± 0.95** | 69.7 ± 4.5** | 135.2 ± 1.7** |
| $CCl_4$ + Silymarin (50 mg/kg) **(V)** | 219.2 ± 10.3** | 175..3 ± 5.2** | 6.84 ± 0.71** | 72.9 ± 3.6** | 146.1 ± 5.4** |

**Legend**
Values are expressed as mean ± S.D. for six rats in each group, *P < 0.001 significantly different from control group (Gr. I), **P < 0.001 significantly different from the group treated with $CCl_4$ (Gr. II.); SOD denotes superoxide dismutase, GSH denotes reduced glutathione, GR denotes glutathione reductase and GST denotes glutathione S-transferase.

Histopathological examination of liver sections from control group showed normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein (Fig 1A). Treatment with $CCl_4$ exhibit group showed various degree of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries (1B). Liver section from rats pre-treatment with methanolic fruit extract of *F. indica* (250 mg/kg and 500mg/kg) showed retention of almost normal architecture with mild hepatic inflammation, moderate degree of vesicular steatosis (Fig 1C & Fig 1D). Same findings were observed from silymarin treated rats (Fig 1E). This indicates *F. indica* extract was found to resist the damage to the hepatoarchitecture caused by $CCl_4$ administration. *F. indica* extract showed a sign of protection to these defects and made it almost comparable to the normal control group which was evident by absence of necrosis and vacuoles.

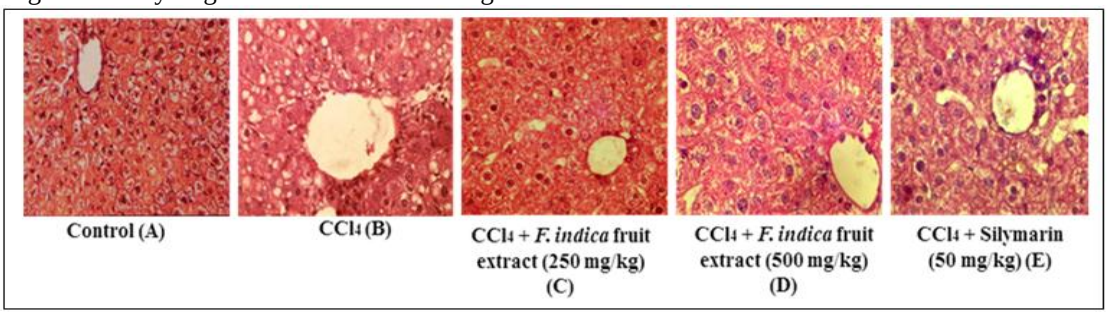


**Figure 1: Histopathological changes of liver tissue treating with *F. indica* fruit extract in paracetamol induced hepatotoxicity in rats**

**Legend**
Representative histopathologic photomicrographs: (A) represents liver from control group rat; (B) represents liver from rat treated with $CCl_4$ treated group; (C) represents liver pretreated with *F. indica* fruit extract at a dose of 250 mg/kg plus $CCl_4$; (D) represents liver pretreated with *F. indica* fruit extract at a dose of 500 mg/kg plus $CCl_4$; (E) represents liver pretreated with silymarin at a dose of 50 mg/kg plus $CCl_4$.

## DISCUSSION

Liver damage induced by $CCl_4$ is commonly used model for the screening of hepatoprotective activity. The rise in serum levels of SGPT, SGOT, ALP, Total bilirubin, and Cholesterol has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages.[3] $CCl_4$ is activated by phase-II detoxifying enzymes in presence of cytochrome P450s in liver cell endoplasmic reticulum to form trichloromethyl and peroxytrichloromethyl free radicals. These can react covalently with several biomolecules such as protein, nucleic acid and lipid proteins in presence of oxygen to induce lipid peroxidation[46,47] resulting in cellular membrane degeneration, increased permeability, and leakage of cytoplasmic ALT, AST and ALP leading to liver damage .[48, 49] Serum levels of ALT, AST and ALP indicates loss of functional integrity of liver cell membrane and should serve as hepatotoxicity indexes.[50] Indeed, CCl4 administration produced significant elevations of serum ALT and AST compared to the normal control group. In the present study, pre-treatment with methanolic extract of *F. indica* (250 mg/kg and 500 mg/kg) attenuated the increases in the activities of SGOT, SGPT, ALP, Total bilirubin, and Cholesterol was found to be lower than the CCl4 treated group indicated that protective effect of *F. indica* against CCl4 induced hepatic damage.

Increased level of lipid peroxidation has been regarded to be an important to produce hepatotoxicity by CCl4.[46,47] In animals treated with methanolic extract of *F. indica* and silymarin, the rise in lipid peroxides in liver tissue homogenate was prevented significantly. The decrease in lipid peroxides may be due to the antioxidant effect of the extract. A possible mechanism of the *F. indica* extract as hepatoprotective may be due to its antioxidant effect or inhibition of cytochrome P450s which impair the bioactivation of $CCl_4$ into their corresponding reactive species.

SOD and Catalase are most important enzymes in the antioxidant defence system. Decrease in activity of these two enzymes is a sensitive index in hepatocellular damage.[51] (Glutathione (GSH), one of the most abundant tripeptide, non-enzymatic biological antioxidants present in the liver removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. The cellular level of glutathione is maintained by GST and GR and reduction of activities of these enzymes therefore would deplete cellular reduced glutathione level and increase the accumulation of toxic metabolites, facilitating a pro-oxidative state.[52] Pre-treatment with *F. indica* extract protects liver from redox imbalance by preventing inhibition of antioxidant enzymes.

The preliminary phytochemical studies indicated the presence of polyphenolic compounds and their glycosides and coumarins in the methanolic extract of fruits of *F. indica*. The presence of significant amounts of polyphenolic compounds in the fruits has been already reported.[53] Since polyphenolic compounds have been reported to have multiple biological effects, including antioxidant activity,[54] specially coumarins have hepatoprotective activity,[55-57] it may be speculated that these constituents of *F. indica* are responsible for the observed protective effects as antioxidants and radical scavengers.

## CONCLUSION

From our results, it can be concluded that increased liver marker enzymes and lipid peroxidation level and decreased levels of antioxidant enzymes in $CCl_4$ treated rats were due to hepatocellular damage. Methanolic extract of *F. indica* fruit offered protection from $CCl_4$ induced liver damage, where 500 mg/kg dose of *F. indica* fruit extract had shown the most pronounced hepatoprotective effect. However, the protective, curative and antioxidant qualities of *F. indica* fruit extract need to be confirmed by characterizing the active ingredient(s) of this plant as well as its mechanism(s) of action. Further research is sought to explore the exact mechanism of action and phytoconstituents responsible for the pharmacological response.

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