

## Hepatoprotective activity of *Ficus bengalensis* Linn leaves.

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### Abstract

The hepatoprotective potentials of the ethanolic extract of the *Ficus bengalensis* Linn leaves were tested against carbon tetrachloride (CCl<sub>4</sub>) and ethanol -induced liver damage in rats. Changes in the levels of biochemical markers of hepatic injury viz; -aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and total albumin (TA) were determined in both treated and control groups of rats. The effects of the extracts were compared with that of silymarin (50 mg/kg). Phytochemical analysis and acute toxicity studies of the extract were also performed. The results showed that CCl<sub>4</sub> and ethanol elevates the levels of AST, ALT and decreased levels of TP and TA. Treatment with the ethanolic extract of *Ficus bengalensis* (EEFB) 100,200 and 400 mg/kg ameliorated the effects of the hepatoxins and significantly ( $P < 0.05$ ) reduced the elevated levels of the biochemical marker enzymes.

### Key Words

Carbon tetrachloride, hepatic marker enzymes, hepatoprotective activity, *Ficus bengalensis*, EEFB,

### Introduction

In spite of tremendous advances in modern medicine, there are no effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. In absence of reliable liver protective drugs in modern medicine, there are a number of medicinal preparations in ayurveda recommended for the treatment of liver disorders and their usage is in vogue since centuries and are quite often claimed to offer significant relief<sup>1</sup>. *Ficus bengalensis* Linn (Moraceae) known locally as 'vad' is traditionally acclaimed to be very effective in the management of liver diseases. The plant grows in lower Himalayas and is found all over India. Different parts of tree have been found to possess medicinal properties. There are more than 800 species and 2000 varieties of ficus species, most of which are native to the world tropics *Ficus bengalensis* is a very large tree reaching 30 m high, sending down many aerial roots from the branches and thus extending the growth of the tree indefinitely<sup>2</sup>. Locally, the plant is used in the treatment of liver disorders, wounds, diarrhoea, skin diseases haemorrhages and rheumatism<sup>3</sup>. It has been reported that *F.bengalensis* plant is used for anti-inflammatory<sup>4</sup>, anthelmintic<sup>5</sup>, antimicrobial<sup>6</sup>, anti-diarrhoeal<sup>7</sup>, antioxidant<sup>8</sup> and in inflammatory bowel diseases<sup>9</sup>.

### Materials and Methods

#### Collection & Extraction

The leaves of EEFB were collected from Bhor region, Dist. Pune, India in the month of September, 2009 and authenticated from Botanical Survey of India Pune, Maharashtra, and herbarium was deposited (BSI/WRC/Tech/2009/546). The shed dried leaves of *Ficus bengalensis* Linn was coarsely powdered in mixture grinder and was then subjected to exhaustive extraction in Soxhlet apparatus using ethanol (99.9%). Then obtained extract were evaporated at 45 °C, the semisolid mass (EEFB) obtained was stored in air tight container in refrigerator for further use.

#### Experimental animals

Albino Wistar rats weighing between 180– 220 g and Swiss albino mice weighing between 25–35 g was used. Institutional Animal Ethics Committee approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were given pellet food (and water *ad libitum*). All the experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Rajgad Dnyanpeeth's College of Pharmacy, Bhor, Pune, Maharashtra, India (Proposal No. RDCOP/IAEC/2009-10/12).

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### **Acute Oral toxicity Studies**

The acute oral toxicity study was performed as per OECD guideline 423. The administration of EEFB at limit dose of 2000 mg/kg did not showed any signs and symptoms of any toxicity in the animals. At this dose no mortality and morbidity was observed during the 14 days of observational period. Hence doses 100 mg/kg, 200 mg/kg and 400 mg/kg of EEFB were selected for present study.

### **Treatment**

The animals were distributed into six groups consisting of six animals each. The first group served as control, the second and third group received low dose and high dose of ethanolic extract of *Ficus bengalensis* (EEFG) at 100 mg/kg p.o., 200 mg/kg, p.o. and 400 mg/kg, p.o. respectively.

### **Phytochemical screening of EEFB**

Quantitative chemical tests were conducted for the ethanolic extract of leaves of *Ficus bengalensis* to identify the various phytoconstituents like alkaloid, carbohydrates, tannins etc as per Khandelwal<sup>10</sup>.

### **Experimental model**

#### **Evaluation of hepatoprotective activity**

#### **Carbon tetrachloride induced hepatotoxicity<sup>11</sup>**

Male Wistar rats (100-150 g) were divided into six groups (n=6) and treated as follows,

Group I: Normal (Distilled water p.o.)

Group II: CCl<sub>4</sub> control (2 ml/kg, i.p. 2<sup>nd</sup> and 3<sup>rd</sup> day)

Group III: Standard drug (Silymarin 50 mg/kg, p.o.)

Group IV: Ethanolic extract of *Ficus bengalensis* (100 mg/kg, p.o.)

Group V: Ethanolic extract of *Ficus bengalensis* (200 mg/kg, p.o.)

Group VI: Ethanolic extract of *Ficus bengalensis* (400 mg/kg, p.o.)

Ethanolic extract of *Ficus bengalensis* and vehicle were administered orally for 5 days. Hepatotoxicity was induced in group II<sup>nd</sup>, III<sup>rd</sup>, IV<sup>th</sup>, V<sup>th</sup> and VI<sup>th</sup> by injection of CCl<sub>4</sub> (2 ml/kg, 1:1 with Olive oil i.p.) on 2<sup>nd</sup> and 3<sup>rd</sup> day. On 5<sup>th</sup> day, the animals were anaesthetized and blood was collected from retro-Orbital plexus. The blood samples were allowed to coagulate for 30 min at room temperature. Serum was separated by centrifugation at 2000 rpm at room temperature for 15 min and subjected to biochemical estimations viz SGOT, SGPT and Total protein. The livers of all animals were removed and processed for liver weight, liver volume and histopathological investigations.

### **Ethanol induced hepatotoxicity<sup>13</sup>**

Wistar Albino rats of either sex (200–220 g) were divided into six groups (n=6) and treated as follows.

Group I: Normal (Distilled water p.o.)

Group II: Ethanol control (20 % Ethanol 3.76 g/kg/day, p.o.)

Group III: Standard (Silymarin 50 mg/kg p.o.)

Group IV: Ethanolic extract of *Ficus bengalensis* (100 mg/kg, p.o.)

Group V: Ethanolic extract of *Ficus bengalensis* (200 mg/kg, p.o.)

Group VI: Ethanolic extract of *Ficus bengalensis* (400 mg/kg, p.o.)

The vehicle (distilled water), ethanolic extract of *Ficus bengalensis*, 20 % ethanol and silymarin were administered orally for 18 days. On the 19<sup>th</sup> day blood was collected from retro Orbital plexuses. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 40 °C for 15 min. and used for the estimation of various biochemical parameters such as SGOT, SGPT and Total protein. After collection of blood samples, the rats were sacrificed and their livers were excised and the liver weight and volume were determined. Histology of liver was carried out

### **Results and Discussion**

Administration of carbon tetrachloride showed significantly elevated levels of serum enzymes SGOT, SGPT and decreased Total protein level due to its enzymatic activation of CCl<sub>3</sub> free radical, which in turn alters the structure and function of liver cells (Sharma et al., 1994). SGOT is found in the liver, cardiac muscles, skeletal muscles, pancreas, lungs, kidney, brain etc. whereas SGPT concentration is highest in the liver and therefore it appears to be sensitive test to hepatocellular damage. Leakage of large quantities of enzymes into blood stream is often associated with massive necrosis of the liver (Shymal et al., 2006). The increased levels of the serum enzymes such as AST and ALT have been observed in CCl<sub>4</sub> administered rats which indicates increased permeability, damage and necrosis of hepatocytes (Ignacimuthu et al., 2009). The Total protein level can be depressed in hepatotoxic conditions due to defective protein biosynthesis in liver. The CCl<sub>4</sub> intoxication causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the

biosynthesis of protein. CCl<sub>4</sub> induces fatty liver and cell necrosis and plays a significant role in inducing triacylglycerol accumulation, depletion of GSH, increased lipid peroxidation, membrane damage, depression of protein synthesis and loss of enzyme activity<sup>4</sup>. In present study, significant (p<0.001) increase in the activities of SGOT, SGPT and decreased levels of Total protein within 48 hr of exposure to CCl<sub>4</sub> was observed compared with normal control group, indicating considerable hepatocellular injury. Treatment with EEFB (200 & 400 mg/kg) and silymarin 50 mg/kg shows significant decrease in all serum biochemical enzyme levels and increase in Total protein level as compared to CCl<sub>4</sub> control group. In present study significant (P< 0.001) increase in liver weight and liver volume in ethanol control group as compared to normal control group was evident in all the paradigms tested. Treatment with EEFB (200 & 400 mg/kg) and silymarin 50 mg/kg significantly (P< 0.001) decreased the liver weight and liver volume in all the paradigms tested compared with ethanol control group. During hepatic damage, cellular enzymes like SGOT and SGPT present in the liver cells leak into the serum, resulting in increased concentrations<sup>(15)</sup>. Ethanol administration for 18 days significantly increased the SGOT, SGPT level and decreased Total protein level. Treatment with EEFB (200 & 400 mg/kg) and silymarin 50 mg/kg shows significant decrease in all serum biochemical enzyme levels and increase in Total protein level as compared to ethanol control group. The standard drug silymarin has been shown to prevent ethanol induced lipid peroxidation and hepatotoxicity. This effect of Silymarin is attributed to its ability to normalize the levels of the transaminases that are elevated in hepatotoxicity<sup>4</sup>.

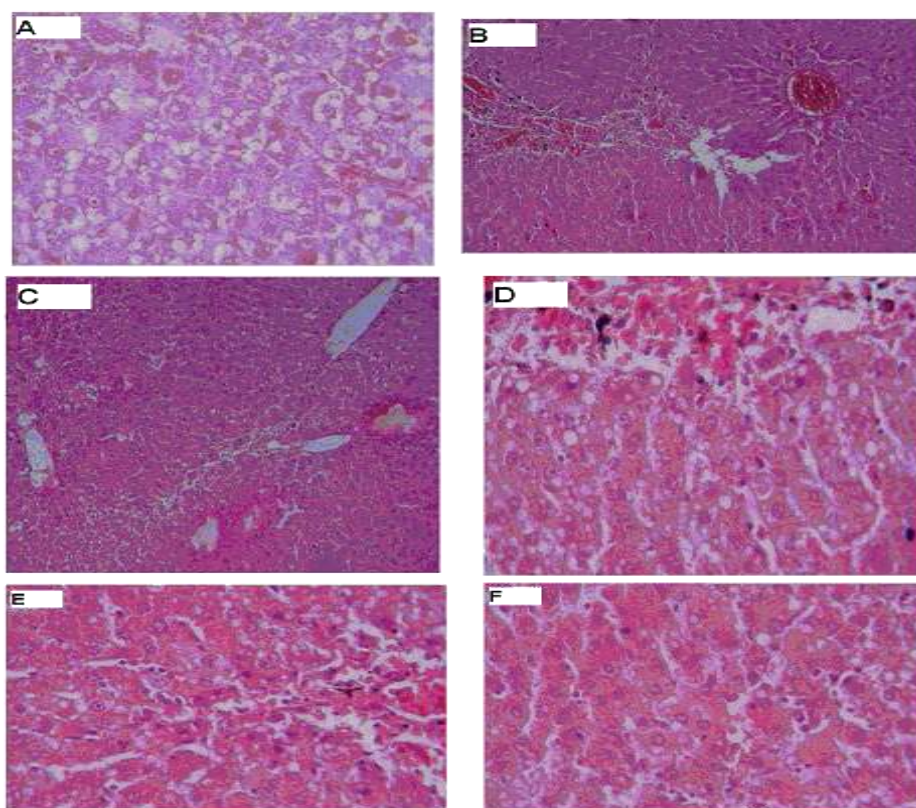
### Conclusion

From the present study, we can conclude that ethanolic extract of *Ficus bengalensis* shows hepatoprotective and antioxidant activity against hepatotoxicants like carbon tetrachloride, paracetamol, ethanol, and rifampicin. Hence EEFB may be act as prophylactic as well as curative drug in treating hepatotoxic conditions. Further studies needs to isolate the active constituents and also to evaluate the exact mechanism of action.

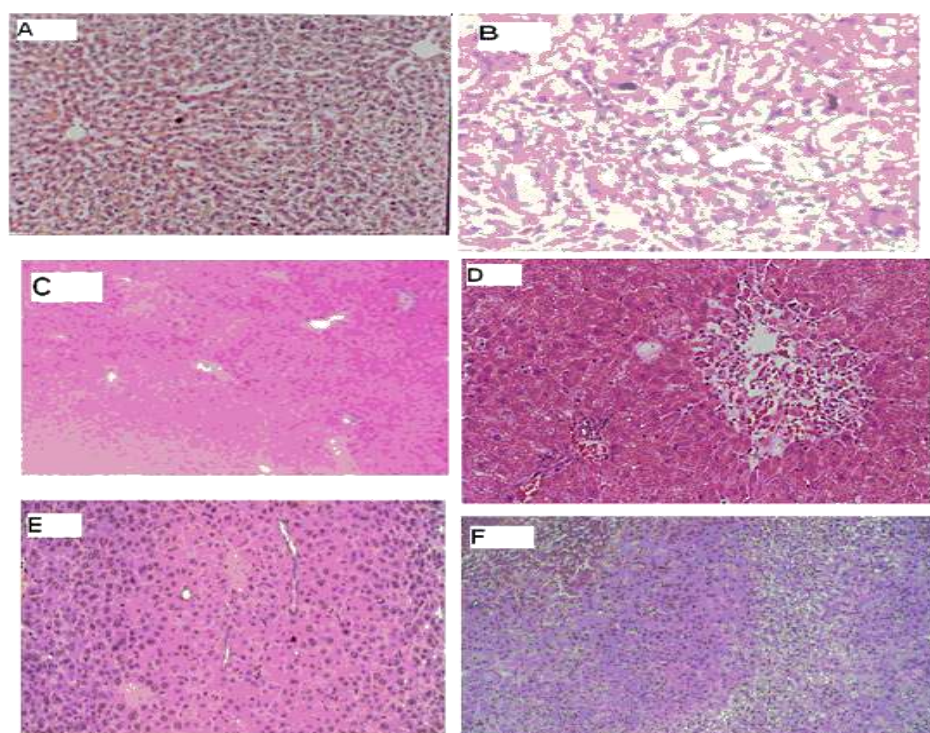
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**Fig. 1:** Photomicrograph of rat liver obtained from different treatment groups (A) Normal control; (B) CCl<sub>4</sub> control; (C) Silymarin; (D) EEFB 100; (E) EEFB 200; (F) EEFB 400 (H & E, 100X).



**Fig. 2:** Photomicrograph of rat liver obtained from different treatment groups. (A) Normal control; (B) Ethanol control; (C) Silymarin; (D) EEFB 100; (E) EEFB 200; (F) EEFB 400 (H & E, 100X).

**Table 1:** Effect of ethanolic extract of *Ficus bengalensis* Linn leaves on physical parameters in carbon tetrachloride induced hepatotoxicity in rats.

Groups	Liver weight (gm per 100 gm)	Liver volume (ml per 100 gm)	SGOT (IU/L)	SGPT (IU/L)	Total Protein (gm/dl)
Normal control	3.94 ± 0.29	4.59 ± 0.12	138.5 ± 12.23	119.4 ± 5.36	6.60 ± 0.27
CCl <sub>4</sub> Control	5.67 ± 0.28 #	5.55 ± 0.16 #	231.0 ± 11.29#	213.8 ± 4.87#	3.91 ± 0.18#
Silymarin 50	4.11 ± 0.23 ***	4.43 ± 0.14 ***	146.5 ± 11.62***	135.0 ± 6.30***	5.72 ± 0.16***
EEFB 100	5.01 ± 0.22	4.78 ± 0.16	186.5 ± 13.48	191.4 ± 5.34*	4.59 ± 0.21
EEFB 200	4.92 ± 0.29*	4.68 ± 0.13*	173.8 ± 12.43*	165.6 ± 7.87**	5.00 ± 0.21**
EEFB 400	4.77 ± 0.35**	4.53 ± 0.17**	162.0 ± 12.29**	141.9 ± 4.83***	5.46 ± 0.21***

Values are expressed as mean ± SEM (n=6 rats). # P<0.001 as compared with normal control using Student t-test (unpaired). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 when compared with the CCl<sub>4</sub> treated group (one-way ANOVA followed by Dunnett's test).

**Table 2:** Effect of ethanolic extract of *Ficus bengalensis* Linn leaves on physical and biochemical parameters in ethanol induced hepatotoxicity in rats.

Groups	Liver weight (gm per 100 gm)	Liver volume (ml per 100 gm)	SGOT (IU/L)	SGPT (IU/L)	Total Protein (gm/dl)
Normal control	4.52 ± 0.32	4.22 ± 0.27	104.1 ± 4.72	118.1 ± 6.43	6.75 ± 0.32
Ethanol control	6.06 ± 0.21#	5.90 ± 0.31#	159.2 ± 6.447 #	208.2 ± 6.29 #	4.23 ± 0.37#
Silymarin 50	4.59 ± 0.23***	4.36 ± 0.24***	119.2 ± 7.482***	138.9 ± 9.21***	5.96 ± 0.26
EEFB 100	5.54 ± 0.19	4.87 ± 0.24	132.7 ± 7.693*	189.6 ± 7.98	5.36 ± 0.23*
EEFB 200	4.95 ± 0.21*	4.86 ± 0.23*	129.0 ± 6.68**	164.3 ± 9.89 **	5.45 ± 0.27*
EEFB 400	4.79 ± 0.32**	4.58 ± 0.26**	121.0 ± 3.80***	156.4 ± 9.69***	5.62 ± 0.21**

Values are expressed as mean ± SEM (n=6 rats). # P<0.001 as compared with normal control using Student t-test (unpaired). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 when compared with the ethanol treated group (one-way ANOVA followed by Dunnett's test).

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