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Hepatoprotective Activity of *Ficus Microcarpa* against Chemical Induce Hepatotoxicity in Rodents

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ABSTRACT

The current study sought to determine the hepatoprotective effect of *Ficus microcarpa* L leaf extracts against carbon tetrachloride (CCl4)-induced liver injury in rats. Methanolic and hydroethanolic extracts of leaves were given orally to Wistar albino rats at a dose of 500 mg/kg body weight, with Silymarin acting as the reference. Methanolic and hydroethanolic extracts restored functional parameters, physical parameters, biochemical parameters, and reduced blood enzymes alkaline phosphatase (ALP) and total bilirubin (TBL) in the chosen animal. Among the plant's chemical constituents include alkaloids, flavonoids, glycosides, steroids, terpenoids, phenolics, and saponins. The overall experimental data suggest that bioactive phytoconstituents discovered in the Methanolic and Hydroethanolic extracts of *Ficus microcarpa* L, such as flavonoids and alkaloids, may be responsible for the plant's significant hepatoprotective activity. As a result of the findings, *Ficus microcarpa* L may be used as a hepatoprotective agent.

Keywords

Carbon tetrachloride, Hepatoprotective activity, *Ficus microcarpa L*.

Introduction

Ficus microcarpa L (Kamrup, Pinwal) has been used in traditional and alternative medical treatments for ages. It's a climber of the Menispermaceae family that grows naturally in India and China [1]. *Ficus microcarpa* L has long, filiform, fleshy aerial roots that develop from its branches and impart a succulent quality to the leaves. The leaves range in colour from pale greenies to dark green and curl deeply to the left [2]. Large rosettes-shaped lenticels can be seen dotting the smooth surfaces between the spirals. Because it is bitter, stomachic, diuretic [3,] promotes bile production, induces constipation, soothes dry mouth, stomach discomfort, and vomiting, and avoids and treatments a burning sensation in the digestive tract, the leaves can be used to treat jaundice and other bile-related illnesses. An extract from its leaves can be used to treat dermatological disorders [4, 5]. When combination with other drugs, *Ficus microcarpa* L is used as an antidote for snake bites and scorpion stings [6, 7]. *Ficus microcarpa* L root extract effectively reduced blood glucose and brain lipids in alloxan-induced diabetic rats [8]. *Ficus microcarpa* L has been linked to immune system advantages such as [9]. In one study, goats treated with *Ficus microcarpa* L improved significantly in clinical and hemato-biochemical markers of CCl4-induced hepatopathy. Furthermore, *Ficus microcarpa* L extract has been demonstrated to inactivate Hepatitis B and E surface antigens in vitro [10]. The anti-inflammatory properties of *Ficus microcarpa* L aqueous extract were beneficial in both cotton pellet granuloma and formalin-induced arthritis models [11]. According to a clinical evaluation [12], patients with

rheumatoid arthritis benefitted considerably from a chemical concoction named "Rumalaya" that contains *Ficus microcarpa* L. Liver diseases are an important public health concern. Herbs can help with liver disorders because mainstream medicine lacks effective liver-protecting drugs. Many different herbs and plant products are used to treat liver disorders in India's traditional medicine and ethnomedicine [13]. Because of the severe side effects of synthetic medications, there is a growing interest in assessing the scientific basis for traditional herbal remedies that are thought to have hepatoprotective potential. Although hundreds of plants are used to prevent or treat illnesses around the world, scientific proof in terms of modern medicine is lacking in most cases, which poses a barrier for the use of medicinal plants in modern medicine. However, if you want to use a plant or its active principles, you must now back up your claims with scientific evidence [14]. To examine if methanol and hydroethanol extracts of *Ficus microcarpa* L could prevent or diminish the severity of CCl4-induced liver damage in rats, we tested their efficacy on CCl4-induced liver damage.

Procurement and Authentication of the Plant

Ficus microcarpa L leaves were collected from the surrounding region of Alwar, Rajasthan, and sent to Sunrise University, Alwar, Rajasthan, where they were verified by the Dept. of Botany, Sunrise University, Alwar, Rajasthan.

Preparation of extracts of *Ficus microcarpa L*

500g of powdered *Ficus microcarpa* L leaves were extracted with solvent in a Soxhlet system to increase polarity. Evaporation was used to concentrate the materials [15].

Animals

Wistar albino rats weighing 150-200 g were obtained from the Central Drug Research Institute in Lucknow, India. The animals were fed a normal pellet diet (Hindustan lever Ltd., Bangalore) and were given free access to water. Before use, all of the animals were acclimatised for a week. After careful consideration, the Institutional Animal Ethics Committee authorised the experimental protocols. The medication was administered to the animals using an oral gavage tube. All of the animals were cared for ethically in accordance with CPCSEA requirements, with frequent rat inspections. The laboratory conditions were properly carried out by a certified veterinary practitioner [16].

Chemicals

The chemicals and solvents used were all of analytical grade. Silymarin was got as a complimentary sample from Micro Lbs in Goa, India. Span Diagnostics Ltd., India, provided standard kits for SGOT, SGPT, and ALP, among other things.

Preliminary phytochemical analysis

A preliminary phytochemical investigation was carried out to establish which phytoconstituents were present in each extract.

Toxicity studies

All of the extracts were tested for acute toxicity using the OECD 423 standards [16]. Female albino rats were used in the acute toxicity study. The animals were fasted for 24 hours with just water provided before receiving the extract orally at dosages of 100, 200, and 500mg/kg b.w., and then monitored for toxic symptoms for up to 72 hours. For all extracts, the therapeutic oral dosage was 500 mg/kg body weight.

Carbon tetrachloride induced hepatotoxicity

The rats, both sexes, were divided into six groups of six. (n = 6) [16, 17]

• Group I (Control) was given water (5 mililitres per kilogramme, p.o.) o.d. for nine days.

• Group II (-ve control): water (5 mililitres/kilogram, p.o.) o.d. for nine days, followed by carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) on the seventh day.

• Group III (+ve control): silymarin (25 mg/kg, p.o.) o.d. for nine days, then carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) was given on the seventh day.

• Administration of Group IV and V (Test Sample) ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extracts of *Ficus microcarpa* L o.d. for nine days, while carbon

tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) was supplied on the seventh day.

Blood was taken from animals on the final day by puncturing the retro orbital plexus. At room temperature, blood samples were allowed to coagulate for 45 minutes. Serum was separated by centrifugation at 2500 rpm for 15 minutes at 30°C and used to estimate several biochemical parameters such as SGOT and SGPT [18], ALP [19], serum bilirubin [20], and serum protein [21]. The animals were sacrificed under profound ether anaesthesia after blood samples were collected. Morphological criteria such as animal weight and liver weight have also been employed to assess the drug's protective impact. Rats with hepatoprotective chemicals lose liver weight/100 gm body weight [22, 23].

Histopathology studies

A piece of liver tissue from each animal group was removed and washed with normal saline. The liver tissues were fixed in 10% buffered neutral formalin for 48 hours, then in bovine solution for 6 hours before paraffin embedding. Sections of 5 mm thickness were cut with a microtome and stained with hematoxylin and eosin. These sections were examined under a 100X magnification light microscope [24].

Statistical Significance

The study's findings were presented as mean SEM, n=6. To analyse and evaluate the data, ANOVA [25] was employed, followed by Dunnet's [26] test for multiple comparisons.

Results

Chemical analysis revealed the presence of phytoconstituents in the various extracts. The results show that ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extracts of *Ficus microcarpa* L leaves have the highest concentrations of pharmacologically active substances such as glycosides, sponins, phytosterols, and flavonoids. As a result, these extracts were selected for pharmacological study. Table 1 summarises the findings. At a dose level of 5000 mg/kg, there was no mortality among the graded dose groups of animals, and they showed no toxicity or behavioural abnormalities. This data indicated that ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extracts of *Ficus microcarpa* L leaves (500 mg/kg) were safe or non-toxic to rats, and so doses of 500 mg/kg, p.o. were used for the investigation. After receiving an intramuscular dose of thiopentone sodium (40 mg/kg), all groups of animals tested fell asleep. When rats were given CCl4, the onset of sleep was significantly delayed (measured in seconds) and the total amount of time spent sleeping was enhanced (measured in minutes). When compared to a CCl4 treatment group, pretreatment with ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extract of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) and silymarin significantly improved sleep onset but considerably reduced sleep duration in rats. Table 2 displays the results. The CCl4-treated group experienced a rise in liver weight and volume, indicating that their livers had expanded in size. ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extracts of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) coupled with silymarin effectively recovered liver weight. Table 3 summarises the findings. Pretreatment with ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extract of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) and silymarin (25 mg/kg, p.o.) demonstrated an ability to mitigate hepatotoxicity by reducing blood marker enzymes. Table 4 summarises the findings. There was a significant rise in total bilurubin and a significant decrease in total protein content in CCl4-treated groups. Pretreatment with ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extract of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) resulted in a substantial decrease in total bilirubin and an increase in total protein. Table 4 summarises the findings.

Histopathological investigations of the liver similarly supported the biochemical analyses. Steatosis (fatty alterations in hepatocytes) and perivenular fibrosis were detected in the CCl4-treated (toxic) control group. These histological alterations were avoided by both extracts.The results were showed in **Figure 1.**

Table 1: Preliminary Phytochemical studies of Extracts of *Ficus microcarpa L* Leaves

Constituents	*Methanolic Extract (MEFM)*	*Hydroethanolic Extract (MEFM)*
Carbohydrate	+	+
Glycosides	-	-
Oil and fats	-	+
Proteins	+	+
Saponins	-	-
Phenolic comp. and tannins	+	+
Phytosterols	+	+
Alkaloids	+	+
Gums and mucilage	+	-
Flavonoids	+	+

Table 2: Effect of *Methanolic (MEFM) & Hydroethanolic (HEEFM)* extract of *Ficus microcarpa L* leaves on functional parameters in CCl_4 induced hepatotoxic rats.

Treatment/ Dose	Onset of sleep(Sec.)	Duration of sleep (Min.)
Normal	177.0 ± 2.16	113.24 ± 2.70
Induced (CCl_4)	79.9 ± 4.78*	255.2 ± 4.80*
Standard (Silymarin)	161.5 ± 4.08***	121.2 ± 4.79***
METC (500 mg/kg)	143.5 ± 4.40**	217.2 ± 4.18**
HEETC (500 mg/kg)	141.4 ± 5.02***	188.2 ± 4.06***

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with CCl_4 induced group and * P<0.05, when compared with normal group.

Table 3: Effect of *Methanolic (MEFM) & Hydroethanolic (HEEFM)* extract of *Ficus microcarpa L* Leaves on Physical Parameters in CCl_4 induced hepatotoxic rats.

Treatment/ Dose	Liver Weight	Liver Volume
Normal	6.25 ± 0.27	6.59 ± 0.28
Induced (CCl_4)	10.12 ± 1.06*	10.02 ± 2.01*
Standard (Silymarin)	6.90 ± 1.18***	7.10 ± 1.73***
METC (500 mg/kg)	8.29 ± 1.10**	8.30 ± 1.41**
HEETC (500 mg/kg)	7.60 ± 1.08* **	7.82 ± 1.59***

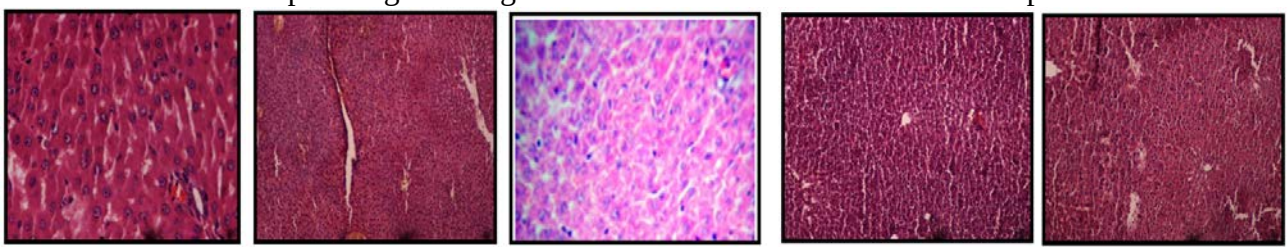
Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with CCl_4 induced group and * P<0.05, when compared with normal group.

Table 4: Effect of *Methanolic (MEFM) & Hydroethanolic (HEEFM)* extract of *Ficus microcarpa L* leaves on serum enzyme parameter in CCl_4 induced hepatotoxic rats.

Treatment/ Dose	SGPT U/L	SGOT U/L	ALP U/L	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
Normal	63.13 ± 3.61	165.25 ± 2.90	184.01 ± 8.12	0.43 ± 0.87	9.64 ± 0.29
Induced (CCl_4)	189.43 ± 8.73*	397.11 ± 8.42*	387.52 ± 7.15*	6.68 ± 7.94*	4.34 ± 0.17*
Standard (Silymarin)	70.56 ± 3.14***	175.48 ± 4.75***	192.41± 8.20***	0.44 ± 2.87***	9.78 ± 4.78***
METC (500 mg/kg)	91.76 ± 3.56***	258.81 ± 4.36***	230.41± 6.71***	0.64 ± 4.98***	7.76 ± 4.50**
HEETC (500 mg/kg)	81.76 ± 5.85***	191.54 ± 8.45***	214.25 ± 8.75***	0.65 ± 0.09	8.67 ± 0.66

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with CCl_4 induced group and * P<0.05, when compared with normal group.

Figure 1: Effect of *Methanolic (Mefm) & Hydroethanolic (Heefm)* Extract of *Ficus Microcarpa L* Leaves on Histopathological Diagram of Liver Tissue in Ccl4 Induced Hepatotoxic Rats.



NORMAL CONTRO **SILYMARIN (25 mg/kg)** **CCL_4 INDUCED** ***METC*** **(500 mg/kg)** ***HEETC*** **(500mg/kg)**

Discussion

Chemicals and medicines are two of the many causes that can cause liver damage or injury. Because it is clinically relevant, CCl4 was employed to produce hepatotoxicity in the current investigation. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) elevations are indicators of hepatocellular damage [27].

CCl4 is metabolised in human cells (endoplasmic reticulum and mitochondria) to yield CCl3O-, a reactive oxidative free radical intermediate produced by cytochrome P450. Lipoperoxidation produces nascent oxygen O-, which causes a rise in intracellular reactive Fe+2 ions, aldehyde, GSH depletion, and calcium restoration. In addition to direct covalent interaction, oxidative CCl3 O- induces Ca+2 sequestration degeneration. Failure to sequester results in increased intercellular Ca+2, proteolytic enzyme aggregation, and an increase in Fe+2 ions, which precipitates aldehyde cytotoxicity via lipid peroxidation [28].

The administration of CCL4 to experimental animals resulted in a statistically significant increase in the levels of enzymes such as SGOT, SGPT, ACP, ALP, and others, indicating chemical-induced hepatocellular toxicity. The inhibitory impact of ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extracts of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) extracts on hepatotoxicity was compared to that of the positive control group. Pretreatment of the animals with the ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extract of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) provided considerable protection in biochemical parameters such as SGOT, SGPT, ACP, and ALP against CCl4-induced elevations. Furthermore, there was an increase in the weight of the liver treated with CCl4 when compared to the control. The ***Methanolic (MEFM) & Hydroethanolic***

(HEEFM) extract of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) treatment keeps the liver weight close to normal. A control rat's liver segment reveals a normal hepatic architectural wall caused by the central vein. The liver samples of CCl4-treated rats showed gross necrosis of the centrilobular hepatocytes characterised by gross necrosis, degeneration, karyolysis, and eosinophilic infiltration, which is significantly prevented by treatment with the hepatoprotective ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extract of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.). The histological pattern of the livers of rats treated with CCl4 plus extracts revealed minimal necrosis in the centrilobular region and hepatocyte regrowth. A number of scientific studies have found that some flavonoids, triterpenoids, and steroids have antioxidant characteristics that protect the liver. Administration of ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extract of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) that demonstrated significant hepatoprotective activity; while qualitative phytochemical investigations on the ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extract of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) that demonstrated positive flavonoids by chemical tests. Furthermore, it has been observed that the plant's flavonoid contents have antioxidant qualities and have been proven to be effective in the treatment of liver damage. [29]

Hepatoprotective medications may cause hepatocytes to withstand the harmful effects of CCl4. The ***Methanolic (MEFM) & Hydroethanolic (HEEFM)*** extracts of *Ficus microcarpa* L Leaves (500 mg/kg, p.o.) showed considerable hepatoprotective action. The results showed that there was a significant level of protection against the hepatotoxic effect of CCl4.

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