Protective Effect of Leaves of *Ficus carica* Against Carbon Tetrachloride-Induced Hepatic Damage in Rats

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**Abstract**

Fig leaves (*Ficus carica Linn.*) belonged to the family Moraceae and used as a source of medicines for the ailments of diseases. The present study was conducted to evaluate the hepatoprotective activity against the carbon tetrachloride induced toxic chemical in rats. The petroleum ether, ethyl acetate and methanol extract of *Ficus carica* leaves was prepared, and evaluated for phytochemical screening. The serum level of glutamic oxaloacetate transaminase (SGOT), glutamic pyruvic transaminase (SGPT) and bilirubin were investigated for the assessment of hepatoprotective activity of ethyl acetate extract. Additionally, the histological changes in liver were observed. Preliminary phytochemical investigations of the extracts of leaves of *Ficus carica* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. Pre-treatment with ethyl acetate extract of *Ficus carica* led to significant (p<0.05) decrease in serum SGOT, SGPT and bilirubin when compared to control group rats treated CCl₄ in dose-dependent manner. The outcomes of histological study revealed that there was significant reversal of histological functional of liver. In conclusion, the findings of this study validated that the *Ficus carica* can improve CCl₄-induced hepatotoxicity.

**Keywords:** *Ficus carica*, Liver, Hepatoprotective activity, Carbon Tetrachloride

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1 Introduction

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions¹. Liver has a wide range of functions, including detoxification, plasma protein synthesis and production of biochemicals necessary for digestion. Damage to the liver inflicted by hepatotoxic agents is of grave consequence. Today, liver damage is one of very common aliment in the world resulting serious debilities ranging from severe metabolic disorders to even motility². Liver functions as a centre of metabolism of nutrients such as carbohydrate, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body there by providing protection against foreign substances by detoxifying and eliminating them³. Liver cells possess the antioxidant defense system consisting of antioxidants such as GSH, ascorbic acid, and vitamin E and antioxidant enzymes such as SOD, catalase, and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death⁴. Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances⁵.

Carbon tetrachloride (CCl₄) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatoprotective effect of CCl₄ is largely due to its active metabolite, trichloromethyl radical. The administration of CCl₄ in rats enhances hepatic protein oxidation and results in the accumulation of CCl₄ oxidized proteins in the liver⁶. Unfortunately, conventional or synthetic drugs used in the treatment of liver disease are inadequate and sometimes can have serious side effects. This is one of the reason for many
people in the world over including those in developed countries turning complementary and alternative medicine. Many traditional remedies employ herbal drugs for the treatment of liver ailments\textsuperscript{7}. In the absence of a reliable liver protecting drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders\textsuperscript{8}.

*Ficus carica* (commonly known as Fig / Anjeer) constituted one of the largest genera of medicinal plants with about 750 species of woody plants, trees, and shrubs primarily occurring in subtropical and tropical regions throughout the world. The genus is remarkable for the large variation in the habits of its species\textsuperscript{2}. The genus *Ficus* (Moraceae) was first published in Systema Naturae by Carolus Linnaeus in 1735. *Ficus* is one of the largest genus among angiosperms. Among the genera of seed plants it ranked as the twenty-first\textsuperscript{16}. It is a small or moderate sized deciduous tree, 3-10 m high with broad ovate or nearly orbicular leaves, more or less deeply 3-5 lobed, rough above and pubescent below; fruits axillary, usually pear shaped, variable in size and colour. The fruit of *Ficus carica* like those of other species of *Ficus*, is a syconium a fleshy hollow receptacle with a narrow aperture at the tip. The bark is a cylindrical and pale grey coloured\textsuperscript{11}. The plant leaves reported to contain furanocoumarins such as psoralen, bergapten, xanthotoxin\textsuperscript{12}, triterpenes such as calotropenyl acetate, lupeol acetate\textsuperscript{13}, isoschaftoside\textsuperscript{14} and certain sterols. *Ficus carica* leaves have been traditionally used in the treatment of vitiligo, diabetes, coughs, asthma, constipation and gingivitis\textsuperscript{15}. The other reported pharmacological activities of fig leaves include cytotoxic\textsuperscript{16}, hypoglycemic\textsuperscript{17} and anthelmintic activity\textsuperscript{18}. Since free radical scavenging and anti-inflammatory activities are crucial factors in management of liver damage, so this plant is suggested to be an efficient hepatoprotective agent. In our study, the fruit extract was tested for hepatoprotective activity against liver damage induced by acetaminophen in rats. Assessment of liver function was performed by determination of its specific serum markers as well as oxidative stress. The present study investigates the hepatoprotective activity of the *Ficus carica* leaves against CCl\textsubscript{4}-induced toxicity in rats as the animal model.

# 2 Materials and Methods

## 2.1 Preparation of plant material

The leaves of the plant of *Ficus carica* were collected from the local surroundings at Kunzer area of Baramulla, Jammu and Kashmir during the month of August and September 2014. The plant was authenticated by Dr. Bikrama Singh, Scientist at taxonomy department Indian Institute of Integrative Medicine (IIIM-CSIR) Jammu and Kashmir, India. The voucher specimens (RRL-22990) are kept in the herbarium of Indian Institute of Integrative Medicine (IIIM)-CSIR Jammu and Kashmir for future reference. The fresh leaves of *Ficus carica* were collected and washed thoroughly under running tap water. The leaves were allowed sun dried after rinsed with distilled water. The dried plant material were coarsely powdered and subjected to extraction.

## 2.2 Preparation of extract

The extract was done by maceration using petroleum ether, ethyl acetate and methanol. The extracts obtained were evaporated in rotary evaporator to get a powdery mass. The powder extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extracts\textsuperscript{19}.

## 2.3 Animals

Male Wistar rats (135-180 g) were used for evaluation of hepatoprotective activity. The animals were housed in polypropylene cages at 25 °C ±1 °C with the relative humidity of 55 ± 5% under 12 h/12 h light/dark cycle. They were received a standard chow and water ad libitum during experimentation. The food was withdrawn on the day before the experiment, but free access of water was allowed. A minimum of six animals was used in each group. Throughout the experiments, animals were process according to the suggested international ethical guidelines for the care of laboratory animals. The study protocol was approved by the Institutional Animal Ethics Committee according to the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals.

## 2.4 CCl\textsubscript{4} induced hepatotoxicity

Assessment of hepatoprotective activity was carried out on wistar albino rats. The animals were segregated into four groups of six animals each. Group I served as normal control receiving 5% CMC (10 ml/kg). Group II received CCl\textsubscript{4} (1 ml/kg i.p.) with equal volume of olive oil (50% v/v) for two successive days, and were maintained as CCl\textsubscript{4} group. Group III animals were animals were treated orally for seven days with suspension of ethyl acetate extract (100 mg/kg). Group IV animals were treated with CCl\textsubscript{4} and ethyl acetate extract (100 mg/kg). After the treatment, the blood samples were collected via retro orbital, and serum was separated by centrifugation at 2500 rpm for 15 min, used for the estimation of biochemical marker enzymes. After collecting the blood, the liver were separated, and weighed\textsuperscript{20-22}.

## 2.5 Biochemical determination

Biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum bilirubin were determined\textsuperscript{23,24}.

## 2.6 Histopathology

Liver were excised quickly fixed in 10% buffered neutral formalin and proceeded for histopathology, they were processed for paraffin embedding following the standard
microtechnique. Sections of liver stained with alum-haematoxylin and eosin were observed microscopically for histopathological changes. A few photomicrographs of representative types were also taken25.

2.7 Statistical analysis

The results are expressed as mean ± SEM of six animals from each group. The data were evaluated by one-way ANOVA followed by Turkey’s multiple comparison tests. P values <0.05 were considered statistically significant.

3 Results and Discussions

3.1 Phytochemical screening of Ficus carica

Presence of classes of secondary metabolite may be a useful indicator of both efficacy and potential toxicity; hence test for the presence of phytochemical classes with known bioactivity was done.

Preliminary phytochemical investigations of the extracts of leaves of Ficus carica revealed the presence of flavonoids,

Table 1: Effect of ethyl acetate extract of Ficus carica on CCL4 induced hepatotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bilirubin (mg/100 ml of blood)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>Liver (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>CMC</td>
<td>0.81±0.06</td>
<td>58.24±2.71</td>
<td>135.12±3.41</td>
</tr>
<tr>
<td>Control (CCL4 1ml/kg i.p)</td>
<td></td>
<td>3.41±0.02a</td>
<td>243.18±6.04a</td>
<td>384.56±5.98a</td>
</tr>
<tr>
<td>Ethyl acetate extract (100 mg/kg oral)</td>
<td></td>
<td>0.76±0.07</td>
<td>63.47±3.42</td>
<td>145.17±4.23</td>
</tr>
<tr>
<td>Ethyl acetate extract (100 mg/kg oral) + CCL4 (1ml/kg i.p)</td>
<td></td>
<td>0.89±0.14b</td>
<td>60.29±5.36b</td>
<td>151.73±4.92b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 when compared with normal control group, **P<0.05 when compared with CCL4 treated group.

Treatment with ethyl acetate extracts of Ficus carica at the dose (100 mg/kg) decreased the activity of SGOT, SGPT and total bilirubin in CCL4 induced liver damaged rats compared to that of CCL4 treated groups (P<0.05). It was found that the test samples offer protection against toxin as evidenced by remarkable reduction in all serum enzyme (P<0.05), and depicted that ethyl acetate extract has strong hepatoprotective action. Further the rats treated only with ethyl acetate extracts of Ficus carica exhibited no significant changes in level of SGOT, SGPT and total bilirubin compared to normal group (Table 1). The increase in weight of liver of CCL4 treated group was observed, and it indicates the fatty liver due toxic effect of CCL4. The extract treated group demonstrated reduction in liver weight, and confirming the protective effect of extract (Table 1).

In the assessment of liver damage by CCL4 hepatoxin, the determination of enzyme level such as SGPT and SGOT are largely used. Necrosis or membrane damage release the enzyme in to circulation, therefore it can be measured in serum. Higher level of SGOT indicates the liver damage, due to active metabolite trichloromethyl free radical produced from carbon tetrachloride during metabolism by hepatic microsomes which in turn cause peroxidation of lipid of cellular membrane. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury23,24. Our results using the model of CCL4-induced hepatotoxicity in the rats demonstrated that ethyl acetate extracts of Ficus carica caused significant inhibition of SGPT and SGOT levels. The bilirubin levels on other hand related to the function of hepatic cells. Increase in serum level of bilirubin was due to increased synthesis, in presence of increasing biliary pressure. Our results using the model of CCL4-induced hepatotoxicity in rats demonstrated that Ficus carica extracts caused significant inhibition of bilirubin levels. Effective control

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of bilirubin level point towards on early improvement in the secretory mechanism of the hepatic cell.

3.3 Histopathological study

In the histopathological study of liver from group I and group III animals showed a normal hepatic architecture (Fig 1a & 1c). In CCl₄ causes focal necrosis, portal infiltration, fatty changes, kupfer cell hyperplasia, hydropic change. In group IV animals, the necrosis which is markedly prevented (Fig b). Milder form of injury like fatty change and reduced necrosis persisted by the extract (Fig d). The toxin mediated histological changes in the liver section of rats of test groups were much less intensity than those observed in the rats of CCl₄ treated group.

Histopathological studies showed that CCl₄ caused focal necrosis, portal infiltration, fatty changes, Kupfer’s cells hyperplasia and hydropic changes of the liver tissue. After administration of ethyl acetate extracts exhibited protection, this confirmed the results of biochemical studies.

Based on our experimental findings, Ficus carica leaves has a protective effect against hepatotoxic CCl₄. The hepatoprotective effects of Ficus carica are likely related to the free radical scavenging effect, which increases antioxidant activity, against membrane lipid peroxidation, and protects membrane integrity and function of liver cells. Further studies are needed to investigate the molecular mechanism of the hepatoprotective effect of Ficus carica.

Fig. 1a: Liver tissue of control rats showing normal histology

Fig. 1b: Liver tissue of CCl₄ treated rats showing necrosis of the hepatic cells

Fig. 1c: Liver tissue of Ficus carica extract (100 mg/kg) treated rats showing no necrosis

Fig. 1d: Liver tissue of CCl₄ + Ficus carica extract (100 mg/kg) treated rats showing necrosis of the hepatic cells is almost prevented

4 Conclusion

Our study suggested a significant protective effect of Ficus carica extract against CCl₄-induced hepatotoxicity. Ficus carica extract exerts this protection through amelioration of lipid peroxidation by its scavenging activity of free radicals and enhancement of the antioxidant defense system.

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6 Conflict of interests

No conflict of interest among all authors of this work

7 Author’s contribution

Research was designed by MS and AR. AR and YA handled data analysis, while NGN handled manuscript writing and revising of content. All authors read and approved the final copy for publication.

8 References
