HEPATOPROTECTIVE ACTIVITY OF FICUS CARICA LINN STEMS EXTRACT AGAINST CARBON TETRACHLORIDE LIVER DAMAGE IN WISTAR RATS.

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ABSTRACT

The ethanolic extract of the stem of Ficus carica linn. (Moraceae) was evaluated for hepatoprotective activity in wistar rats with liver damage induced by carbon tetrachloride. The extract at an oral dose of 500 mg/kg exhibited a significant protective effect by lowering the serum levels of aspartate aminotransferase, alanine aminotransferase, total serum bilirubin and malondialdehyde equivalent, an index of lipid peroxidation of the liver. These biochemical observations were supplemented by histopathological examination of liver sections. The activity of extract was also comparable to that of silymarin, a known hepatoprotective.

KEYWORDS: Ficus carica linn, Stems, Hepatoprotective; Carbon tetrachloride.

INTRODUCTION

Liver is the largest organ in the vertebrate body and the site for intense metabolism. Liver diseases remain one of the serious health problems and the Indian traditional system of medicine, especially Ayurveda have put forward a number of medicinal plants and their formulations for liver disorders. In this modern age it is very important to provide scientific proof to justify the various medicinal uses of herbs. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost. However, we are not aware of a satisfactory remedy for
seriously liver diseases and search for effective and safe drugs for liver disorders continues to be an area of interest.

Egypt one of countries suffering from hepatitis C virus (HCV) in the world, with an estimated 8–10 million among a population of 68 million having been exposed to the virus and 5–7 million active infections (Frank et al., 2000).

Carbon tetrachloride is one of the most widely used hepatic toxins for experimental induction of hepatic fibrosis and cirrhosis in experimental animals(Jiang et al., 2004; Singab et al., 2005). CCl4 is frequently found as a byproduct in drinking water, which remains a potential health hazard to humans. Furthermore, most CCl4 in food is residual contamination fumigation as a pesticide (Weber et al., 2003). CCl4 prompted cirrhosis or fibrosis in experimental animals resembles cirrhosis of human in some features of morphology and pathophysiology. CCl4 are widely used as model compound to induce hepatotoxicity and elucidate its mechanisms of action following exposure to these compounds (Kim et al., 2010).

There has been a great deal of interest recently in role of alternative and complementary medicines for the treatment of various acute and chronic diseases (see et al., 2001).

Ficus carica Linn. has been used for metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory disorders (Patil Vikas, et al., 2010). It has high minerals, vitamins, dietary fibers and phenolic contents which play an important role in its antioxidant capacity (Veberic, et al., 2008).

Analysis of antioxidants in fig revealed that it contains significant amounts of the antioxidant vitamins; vitamin A and vitamin C. Lattanzio, (2003) reported that besides antioxidant effects, phenolic compounds have a wide variety of biochemical properties and can also have a useful effect in preventing the development of ailment like cancer and cardiovascular diseases. There is linear correlation between the total content of phenolics and the antioxidant capacity (Cai et al., 2004; kumaran and karunakaran, 2006).

Ficus carica L., (Moraceae) is a deciduous tree, which grows in tropical and subtropical regions of India. F. carica L. is claimed to be useful in liver and spleen disorders, to cure piles and in treatment of gout. Earlier chemical examination of this plant have shown the presence of 3-O- and 5-O-caffeoylquinic acids, ferulic acid, quercetin-3-O-glucoside,
quercetin-3-O-rutinoside, psoralen, bergapten, and organic acids (oxalic, citric, malic, quinic, shikimic, and fumaric acids). The stems of F. carica consist of various volatile compounds which are identified and distributed by distinct chemical classes, such as aldehydes: methylbutanal, 2-methylbutanal, (E)-2-pentanal, hexanal, and (E)-2-hexanal, alcohols: 1-penten-3-ol, 3-methyl-1-butanol, 2-methylbutanol, heptanol, benzyl alcohol, (E)-2-nonen-1-ol, and phenylethyl alcohol, ketone: 3-pentanone, esters: methyl butanoate, methyl hexanoate, hexyl acetate, ethyl benzoate, and methyl salicylate, monoterpenes: limonene and menthol, sesquiterpenes: α-cubenene, α-guaiene, α-ylangene, copaene, β-bourbonene, β-elemene, α-gurjunene, β-caryophyllene, β-cubebe, campesterol, taraxerone, α-caryophyllene, α-muurolene, germacrene D and (+)-ledene, norisoprenoid: β-cyclotiral, and miscellaneous compounds: psoralen. The present work was mainly conducted to examine the effect of hepatoprotective activity of Ficus carica stems ethanolic extract against carbon tetrachloride induced hepatotoxic damage in male wistar rats the biochemical parameters will be assayed to evaluate the protective effect of Ficus carica stems extract.

MATERIAL AND METHOD

Collection of plant material

The stem of the Ficus carica L., (Moraceae) was collected from Botanical Garden of N.B.R.I (National Botanical Research Institute), Lucknow, India in month of July 2016. The plant materials were authenticated in department of chemotaxonomy at National Botanical Research Institute, Lucknow and voucher specimens were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference.

Preparation of extract

Dried stem powder (800 g) was extracted with ethanol by maceration for five days. The concentrated ethanolic extract (12.5 g) was tested for qualitative phytoconstituents and indicated the presence of steroids/triterpenoids and their glycosides and coumarins.

Animals

Male Wistar rats (160-250 gm.) were procured from National Botanical Research Institute (Lucknow). They were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10%
protein, 4% arachis oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D (Zimmerman M, 1983).

Hepatoprotective effect against CCl₄-induced hepatotoxicity in rats
Animals were divided into four groups of six rats each. Group I and II served as normal and intoxicated control, respectively and received only the vehicle (5% gum acacia; 1 ml/kg; p.o). Group III animals were treated with standard silymarin at an oral dose of 100 mg/kg and group IV received the ethanolic extract of *Ficus carica* L. at an oral dose of 500 mg/kg, as a fine suspension of 5% aqueous gum acacia. The treatment was continued for 7 days, once daily. On the day of 7 for groups II-IV, 30 min post-dose of extract administration animals received CCl₄ at the dose of 1.5 ml/kg (1:1 of CCl₄ in olive oil) orally. The animals were sacrificed after 36 h after administration of acute dose of CCl₄. The blood was collected by carotid artery. The serum was separated out and used for estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total serum bilirubin using Span diagnostic kits. The liver was immediately removed and the liver tissue was used for estimation of malondialdehyde equivalent, an index of lipid peroxides and a section of liver was processed for histological studies.

Histopathological studies
The tissues of liver were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope.

Statistical analysis
The results are expressed as means ±S.D. The difference between experimental groups were compared by one-way ANOVA (toxic control versus treatment, Bonferroni’s method; using Jandal scientific, Sigmastat statistical software, version 1.0) and were considered statistically significant when p< 0.05.

RESULTS
The animals treated with toxic doses of carbon tetrachloride had markedly elevated values of the serum ALT, AST, ALP and total bilirubin compared to normal rats, indicating acute hepato-cellular damage (Table-1). Serum enzyme values in the animals pretreated with ethanolic extract of *F. carica* L., (500 mg/kg; p.o) were significantly (p< 0.001) lower than those of toxic control values and except for ALP. ALT, AST, total bilirubin serum enzyme
values in treated animals were similar to the normal control values. Malondialdehyde values in the *F. carica* L., extract treated animals were significantly lower (p< 0.001) than those of toxic control values. The effects of the ethanolic extract of *F. carica* L., stems were comparable to that of standard silymarin activity.

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig.1). Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in CCl₄ intoxicated animals (Fig. 2). The liver sections of the rats treated with ethanolic extract of *F. carica* L., and silymarin followed by CCl₄ intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Fig. 3 and 4).

**DISCUSSION**

The ethanolic extract of *F. carica* L., stems, administered prophylactically, exhibited significant protection against CCl₄-induced liver injury as manifested by the reduction in toxin-mediated rise in serum transaminases, ALP and total bilirubin in rats. Liver damage induced by CCl₄ is commonly used model for the screening of hepatoprotective drugs. The rise in serum levels of AST, ALT and ALP has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages. The rise in the levels of serum bilirubin is the most sensitive and confirms the intensity of jaundice. The CCl₄ is converted into reactive metabolite, halogenated free radical by hepatic cytochrome P450s, which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation with subsequent tissue injury. High lipid peroxidation values indicate excessive free radical induced peroxidation. The measurement of
lipid peroxide is also a marker of hepatocellular damage.

Pretreatment of animals with ethanolic extract of *F. carica* L. and silymarin prevented the CCl₄-induced rise in serum level of transaminases and total serum bilirubin, confirming the protective effects of ethanolic extract of *F. carica* L., stems against carbon tetrachloride induced hepatic damage. The hepatoprotective activity of *F. carica* L., (500 mg/kg) was comparable with the activity of standard silymarin (100 mg/kg). However there was no effect on rise in serum alkaline phosphatase levels by the test extract and silymarin. A comparative histopathological study of the livers from different groups further corroborated the hepatoprotective potential.

In animals treated with ethanolic extract and silymarin, the rise in lipid peroxides in liver tissue homogenate was prevented significantly. The decrease in lipid peroxides may be due to the anti-oxidant effect of the extract. A possible mechanism of the *F. carica* L., extract as hepatoprotective may be due to its anti-oxidant effect or inhibition of cytochrome P450s which impair the bioactivation of CCl₄ into their corresponding reactive species.

The preliminary phytochemical studies indicated the presence of steroids/triterpenoids and their glycosides and coumarins in the ethanolic extract of leaves and bark of *F. carica* L., Since coumarins have hepatoprotective activity, it may be speculated that these constituents of *F. carica* L., are be responsible for the observed protective effects. However the role of steroids/triterpenoids cannot be ruled out.

**Table 1: Effect of pretreatment with ethanolic extract of *F. carica* L., on CCl₄-induced rats.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment, p.o</th>
<th>Bio chemical parameters</th>
<th>MDA (equi)</th>
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<tr>
<td></td>
<td></td>
<td>ALT (U/L)</td>
<td>AST (U/L)</td>
</tr>
<tr>
<td>I</td>
<td>Control-Normal</td>
<td>23±2.8</td>
<td>129.6±6.0</td>
</tr>
<tr>
<td>II</td>
<td>CCl₄-treated</td>
<td>39.2±1.7</td>
<td>138.2±3.8</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin+CCl₄</td>
<td>25.7±1.9*</td>
<td>127.2±3.4*</td>
</tr>
<tr>
<td>IV</td>
<td>EeOH ext+CCl₄</td>
<td>24.6±2.4*</td>
<td>119.5±4.5*</td>
</tr>
</tbody>
</table>

Groups from II to IV received CCL₄ 30 min after treatment on 7th day.
Values are mean ±S.D; n=6. *p<0.001 vs. Group II
REFERENCES


