Hepatoprotective Effect of *Ficus carica* Leaf Extract on Mice Intoxicated with Carbon Tetrachloride

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**Abstract**

Protective action of *Ficus carica* leaf ethanolic extract (obtained by maceration) was evaluated in an animal model of hepatotoxicity induced by carbon tetrachloride (CCl₄).

Male albino mice were divided into six groups. group I was normal control group; group II received olive oil (CCl₄ solvent), groups III–VI received CCl₄. After inducing hepatic damage, group III served as control for CCl₄; and groups IV–VI received different doses of *Ficus carica* ethanol extract (200, 400 and 800 mg/kg) prior to intoxication with CCl₄. Liver marker enzymes were assayed in serum. Sections of livers were observed under microscope for the histopathological changes. Levels of marker enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were increased significantly in CCl₄ treated mice (group III). In groups IV, V and VI, pre-treated with the plant extract and intoxicated with CCl₄, decreased activities of these two enzymes were observed. Also, pre-treatment with the extract in these groups resulted in less pronounced destruction of the liver architecture with no fibrosis and moderate inflammation was observed compared with group III. The present observations suggested that the treatment with *Ficus carica* leaf extract in dose of 200 mg/kg enhanced protection against CCl₄ induced hepatic damage.

**Keywords:** *Ficus carica;* Liver; Carbon tetrachloride; Hepatoprotective; Mice.

**Introduction**

The genus *Ficus* belongs to the family Moraceae and to the order urticales (1). Moraceae is composed of trees and shrubs which characteristically have a milky juice. *Ficus* constitute one of the largest genera of angiosperms, with almost 800 species of terrestrial trees, shrubs, hemi-epiphytes, climbers and creepers occurring in the tropics and subtropics worldwide (2). *Ficus* are important genetic resources with high economic and nutritional value. They are also an important part of the biodiversity in the rainforest ecosystem by setting fruit throughout the year and providing an important source of food for fruit-eating animals in the tropics.

*Ficus carica* (fig tree) has been extensively investigated for its proteolytic enzymes (3), amino acids, minerals and sugars (4), triterpenes (5), and organic acids (6). The leaves are added to boiling water and used as a steam bath for painful or swollen piles, and as a decoction and stomachic (7). The fruit is mildly laxative, demulcent, digestive and pectoral (8). The leaf decoction is taken as a remedy for diabetes and calcifications in the kidneys and liver (9).

One of the traditional methods for treatment of warts in some rural areas of Iran is to use fig...
tree latex as a local treatment, as reported by Avicenna in his 10th century book *Canon of Medicine* (10).

Despite the fact that other parts of the fig tree, like the fig leaves, have also reported pharmacological properties, they have been much less investigated. In 1998, Serraclara et al. (11) reported the hypoglycemic action of a fig leaf decoction in type-I diabetic patients, and in 2000, Canal et al. (12) used a chloroform extract, obtained also from a decoction of *F. carica* leaves, to decrease the cholesterol levels of rats with diabetes. These pharmacological properties are probably in part due to the high content of phenolic compounds in these plant extracts. Administration of the *F. deltoidea* leaves aqueous extract produced significant dose-dependent antinociceptive effect in animal model (13).

Liver is the first major organ to be exposed to ingested toxins due to its portal blood supply and toxins may be, at least partially, removed from the circulation during the first pass, providing protection to other organs while increasing the likelihood of hepatic injury (14). Liver toxicity is monitored in standard toxicity studies by a range of investigations including clinical biochemistry parameters (enzymes, proteins, lipids, etc.). The following endpoints are considered to be mainly related to liver toxicity: its relative weight and more than two enzymes indicative of hepatocellular effects such as (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase) (15).

Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning complimentary and alternative medicine. Many traditional remedies employ herbal drugs for the treatment of liver ailments (16). A number of plants have been shown to possess hepatoprotective property (17-19).

Considering that other species of *Ficus* had hepatoprotective activity, the present investigation was undertaken to test the efficacy of different doses of ethanolic extract prepared from the leaves of *Ficus carica* against hepatic injury induced by CCl₄ in mice to determine the possible use of this plant in preventing hepatic damage.

**Experimental**

**Materials and methods**

**Animals**

Male albino mice weighing 30-31.5 g were obtained from Animal House of Ahwaz Jundishapour University of Medical Sciences and were kept at 23-25°C, under the light and dark cycles of 12 h, at 60-80% relative humidity, and fed with a standard diet and watered *ad libidum*. All experiments were performed in the morning.

**Chemicals**

Assay kits for the estimation of serum enzymes (ALT and AST) were purchased from pars azmun (liquid test). All other chemicals were of analytical grade.

**Plant material**

Leaves of *Ficus carica* were collected from campus garden of School of Pharmacy in summer of 2007. The plant material was identified and authenticated taxonomically by Dr. Sedigi, Faculty of Agriculture, Shahid Chamran University, Ahwaz, Iran and a voucher specimen was stored in the herbarium of Pharmacognosy Department, School of Pharmacy, Ahwaz Jundishapour University of Medical Sciences, Ahwaz, Iran.

**Preparation of plant extract**

The leaves were shade dried, powdered and then were extracted with 80% aqueous EtOH by maceration at room temperature for 72 h. The extract was filtered and the filtrate was concentrated in a rotary evaporator under reduced pressure.

**CCl₄ induced hepatotoxicity**

Mice were divided into six groups of seven animals in each group. Table 1 shows the dose schedule of carbon tetrachloride and test samples against CCl₄ intoxication. Each mouse was treated for four days with saline (0.9%), olive
oil (CCl\textsubscript{4} solvent); CCl\textsubscript{4} (0.2 mL/kg in olive oil) or test samples (plant extract) orally in the total volume of 0.2 mL. Mice in test groups, in the second and third day, were given CCl\textsubscript{4} half an hour after the administration of the plant extract dose. Liver weight changes, biochemical and histopathological evaluation were undertaken on the fifth day.

Animals were killed in the fifth day by using chloroform, their blood was collected, allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 min. Liver was dissected out and used for histopathological studies.

**Biochemical determination**

The biochemical parameters (serum enzymes): alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed spectrophotometrically using a commercially available assay kits according to the manufacturer’s protocol.

**Liver histopathological assessment**

Liver sections, taken immediately from liver, fixed in 10\% formalin, dehydrated in gradual ethanol (50-100\%), cleared in xylene and embedded in paraffin. Sections (4-5 \textmu m thick) were prepared and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic observation, including cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, infiltration of Kupffer cells and lymphocytes.

**Statistical analysis**

The data are expressed as mean ± SD. Data were analyzed by analysis of variance (ANOVA) followed by multiple comparisons using Tukey test to compare all groups against control. Results were considered statistically significant at p < 0.05.

**Results and Discussion**

Liver is a major site for metabolism of exogenous chemicals (pesticides, drugs, metals), resulting in the formation of metabolites which may be more or less toxic than the parent compound (20). CCl\textsubscript{4}, an extensively studied liver toxicant, and its metabolites such as CCl\textsubscript{3} radicals are known to be involved in the pathogenesis of liver damage.

The yield (w/w) of the ethanolic extract was 6.36\%. No mortality observed in any of the groups. The results of hepatoprotective effects of different *Ficus carica* leaf extracts on CCl\textsubscript{4} intoxicated mice are shown in Table 2. There was no significant changes in the activities of serum ALT, AST and liver weight in group of olive oil mice compared to negative control group (Table 2). The liver weights of the three Test groups calculated at the end of the study had decreased (statistically significant) when compared with CCl\textsubscript{4} induced group (Table 2). In the three test groups the level of marker enzymes were found retrieving towards normalcy.

The histopathological observation basically support the results obtained from enzyme assays. Histology study of liver from negative control group showed a normal hepatic architecture (Figure 1). In CCl\textsubscript{4} induced group, severe hepatotoxicity (massive fatty changes, necrosis,
ballooning degeneration and broad infiltration of the lymphocytes and Kupffer cells around the central vein) was seen (Figure 2). The histological architecture of liver sections of mice treated with different *Ficus carica* leaf extracts showed a more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the normal control group (Figure 3).

In previous investigations, hepatoprotective effect of leaf extracts of other species of *Ficus* were examined. Mandal et al. showed that an oral dose of 400 mg/kg of *Ficus hispida* methanolic leaf extract exhibited a significant protective effect against paracetamol-induced hepatotoxicity in rats (21). The hepatoprotective activity of *Ficus racemosa* leaf extract on liver damage caused by carbon tetrachloride in rats was comparable to standard liver tonic (22).

In another study, carried out by Krishna Mohan et al., the protective effect of methanolic leaves extract of *Ficus carica* was reported at a dose of 500 mg/kg (23). In the present research, we tried three different doses and obtained a significant liver protection at dose of 200 mg/kg.
Table 2. Effect of different Ficus carica leaf extract on CCl₄ induced rise in serum ALT, AST and changes in liver weight in mice.

| Groups (n = 7)         | ALT (IU/L) (Mean ± SEM) | AST (IU/L) (Mean ± SEM) | Liver weight (g) (Mean ± SEM) |
|------------------------|-------------------------|-------------------------|-------------------------------|
| Negative Control       | 116.75 ± 1.60           | 138.15 ± 5.05           | 1.60 ± 0.035                  |
| Olive Oil              | 117.14 ± 2.99           | 145.28 ± 2.95           | 1.41 ± 0.025                  |
| CCl₄                   | 190.90 ± 1.35*          | 239.40 ± 2.84*          | 1.89 ± 0.079*                 |
| Test 1                 | 85.57 ± 4.23*           | 194.71 ± 9.88*          | 1.22 ± 0.074*                 |
| Test 2                 | 96.71 ± 2.99*           | 204.00 ± 14.86*         | 1.50 ± 0.084*                 |
| Test 3                 | 132.43 ± 15.76*         | 224.00 ± 11.21          | 1.50 ± 0.081*                 |

Negative control received normal saline, Test 1, 2 and 3 received 200, 400 and 800 mg/kg of Ficus carica leaf extract, respectively. p* < 0.05 as compared with CCI₄ induced group. p < 0.05 as compared with negative control group.

References

(1) Hutchinson J. The Families of Flowering Plants. 2nd ed., Vol. 1, Clarendon Press, Oxford (1959) 201.
(2) Frodin DG. History and concepts of big plant genera. Taxon (2004) 53: 753-776.
(3) Oner MD and Akar B. Separation of the proteolytic enzymes from fig tree latex and its utilization in gaziantepe cheese production. Lebensm-Wiss. Technol. (1993) 26: 318-321.
(4) Kim SS, Lee CH, Oh SL and Chung DH. Chemical components in the two cultivars of Korean figs Ficus carica L. J. Korean Agric. Chem. Soc. (1992) 35: 51-54.
(5) Ahmed W, Khan AQ and Malik A. Two triterpenes from the leaves of Ficus carica. Planta Med. (1988) 54: 481-488.
(6) Shiraiishi SC, Kawakami K, Widodo SE, Shiraiishi M and Kitazaki M. Organic acid profiles in the juice of fig fruits. J. Fac. Agric. Kyushu Univ. (1996) 41: 29-33.
(7) Duke JA and Ayensu ES. Medicinal Plants of China. Vol. 2, Reference Publications, Michigan (1985) 390.
(8) Chiej R. Encyclopedia of Medicinal Plants. McDonald Publishers, London (1984).
(9) Morton JF. Fig. In: Morton JF. (ed.) Fruits of Warm Climates. Creative Resources Systems Inc., Winterville (1987) 47-50.
(10) Hemmatzadeh F, Fatemi A and Amini F. Therapeutic effects of fig tree latex on bovine papillomatosis. J. Vet. Med. B. Infect. Dis. Vet. Public Health (2003) 50: 473-476.
(11) Serraclara A, Hawkins F, Perez C, Dominguez E, Campillo JE and Torres MD. Hypoglycemic action of an oral fig-leaf decoction in type-1 diabetic patients. Diabetes Res. Clin. Pract. (1998) 39: 19-22.
(12) Canal JR, Torres MD, Romero A and Perez C. A chloroform extract obtained from a decoction of Ficus carica leaves improves the cholesterolaemic status of rats with streptozocin-included diabetes. Acta Physiol. Hung. (2000) 87: 71-76.
(13) Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS and Israa DA. Evaluation of the antinociceptive activity of Ficus deltoidea aqueous extract. Fitoterapia (2008) 79: 557-561.
(14) Moslen MT. Toxic responses of the liver. In: Klaassen CD, Amdur MO and Doull J. (eds.) Casarett and Doull's Toxicology, The Basic Science of Poisons. 5th ed., McGraw-Hill, NY (1996) 403-416.
(15) Solecki R, Davies L, Delhícoro V, Dewhurst I, Raaij MV and Trüsscher A. Guidance on setting of acute reference dose (ARfD) for pesticides. Food Chem. Toxicol. (2005) 43: 1569-1593.
(16) Mitra SK, Shashidhri SJ, Venkataranangam MV, Gopumadhav S, Venkatesh Udupa U and Sarma DNK. Effect of HD-03, an herbal formulation in galactosamine-induced hepatopathy in rats. Indian J. Physiol. Pharmacol. (2000) 44: 82-86.
(17) Sehrawat A, Khan TH, Prasad L and Sultana S. Butea monosperma and chemomodulation: protective role against thioacetamide-mediated hepatic alterations in Wistar rats. Phytomedicine (2006) 13: 157-163.
(18) Jamshidzadeh A, Khoshnood M, Dehghani Z and Niknahad H. Hepatoprotective activity of Cichorium intybus L. leaves extract against carbon tetrachloride induced toxicity. Iranian J. Pharm. Res. (2006) 1: 41-46.
(19) Bhandarkar M and Khan A. Protective effect of Lawsonia alba Lam., against CCI₄ induced hepatic damage in albino rats. Indian J. Exp. Biol. (2003) 41: 85-87.
(20) Miyai K. Structural organization of the liver. In: Meeks JF, Harrison SD and Bull RJ. (eds.) Hepatotoxicology. CRC Press, Boston (1991) 1-65.
(21) Mandel SC, Saraswathi B, Kumar CK, Mohana-Lakshmi S and Maiti BC. Protective effect of leaf extract of Ficus hispida Linn. against paracetamol-induced hepatotoxicity in rats. Phytother. Res. (2000) 14: 457-9.
(22) Mandel SC, Maiti TK, Das J, Pal M and Saha BP. Hepatoprotective activity of Ficus racemosa leaf extract on liver damage caused by carbon tetrachloride in rats. Phytother. Res. (1999) 13: 430-2.
(23) Krishna Mohan G, Pallavi E, Ravi Kumar B, Ramesh M and Venkatesh S. Hepatoprotective activity of Ficus carica Linn. Leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. Daru (2007) 15: 162-166.

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