

Effects of Fig tree (*Ficus carica*) leaf extracts on serum and liver cholesterol levels in hyperlipidemic rats

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Abstract

The effects of three *Ficus carica* leaf extracts on the total cholesterol levels (TC) of serum and liver were investigated in experimentally-induced nutritional hyperlipidemic rats. In nine treatment groups (n=5 each group), hyperlipidemic rats were treated daily with hydromethanolic (total) extract (2.5, 5, 10 mg/kg, intraperitoneal [ip]) and its aqueous fractions, namely fraction A (10, 50, 250 mg/kg, ip) and fraction B (10, 50, 250 mg/kg, ip) for eight days. In negative and positive control groups, animals received normal and hyperlipidemic diets with ip injections of normal saline, respectively. The lipid-lowering effect of total extract on liver cholesterol was more pronounced than that of serum. Fraction A caused a significant dose-dependent decrease in cholesterol levels in both the serum and the liver ($p < 0.05$). Fraction A at dosages of 10, 50 and 250 mg/kg lowered the TC in serum from 1.40 ± 0.26 mmol/L (mean \pm standard deviation; untreated hyperlipidemic group) to 1.06 ± 0.14 , 1.04 ± 0.07 and 0.90 ± 0.08 mmol/L, respectively. At 50 and 250 mg/kg, it lowered the TC in liver significantly from 59.86 ± 10.35 mg/g (untreated) to 42.61 ± 12.08 and 37.16 ± 5.59 mg/g, respectively. Fraction B lowered the level of TC in the serum and liver, but the results of this treatment were conflicting. Phytochemical screening showed that total extract had moderate levels of flavonoids and a large amount of tannins, which may account for the observed effects on decreasing TC levels. In conclusion, *Ficus carica* leaf extracts have been shown to decrease liver and serum TC levels in hyperlipidemic rats.

Introduction

Plant materials remain a source of potential for the discovery of new compounds with valuable pharmacological activities. Nowadays, atherosclerosis due to hypercholesterolemia is a major health concern in humans (Perez *et al.*, 1999; Nakamura *et al.*, 2000). Traditional medicine is often pursued as an alternative for conventional drugs for treatment of hypercholesterolemia. To date, many herbal products have been investigated for their therapeutic effects both in humans and in experimental animals. The hypolipidemic effects of some plants have been demonstrated, including *Viccinium myrtillus* (Cignarella *et al.*, 1996), *Allium sativum* (Evans, 2002), *Curcuma xantorrhiza* (Yansi and Imaizmi, 1991), and *Salvadora persica* (Galati *et al.*, 1999).

Moreover, several therapeutic effects have been shown for *Ficus carica* (Fig tree), such as hypoglycemic (Perez *et al.*, 2003; Serraclara *et al.*, 1998), cancer

suppressive (Rubnov *et al.*, 2000), antihelminthic (De-Amorin *et al.*, 1999) and hypolipidemic effects (Perez *et al.*, 1999; Campillo *et al.*, 1994). The effects of *Ficus carica* decoction on postprandial hypertriglyceridemia in rats (Perez *et al.*, 1999) and the effects of *Ficus carica* leaf latex on chicken hepatic cell triglyceride secretion and content have been demonstrated previously (Asadi *et al.*, 2006). The chloroform extract obtained from a decoction of *Ficus carica* leaves has been shown to reduce blood cholesterol levels in streptozocin-induced diabetic rats (Canal *et al.*, 2002).

The aim of the present study was to discern the effect of different *Ficus carica* leaf extracts on the cholesterol levels in the serum and liver in rats with experimentally-induced nutritional hypercholesterolemia.

Materials and Methods

Preparation of *Ficus carica* leaf extracts

The leaves of *Ficus carica* were collected from fig

trees at the Amin-Abad Research Institute, The University of Tehran, Tehran, Iran in July 2005. They were dried by open air flow out of the sunlight, grinded into powder, and kept in glass containers at room temperature ($25\pm 3^{\circ}\text{C}$).

Five different extracts were prepared in the Pharmacognosy Lab, Faculty of Pharmacy, University of Medical Sciences, Tehran, Iran. At first, an 80% hydromethanolic (total) extract was prepared using the suxhlet apparatus (HP-6-500, Aratebfan Ltd., Iran) and a rotary evaporator (Heidolph, Germany). An aliquot of dried total extract was then re-extracted by water-petroleum ether and another aliquot was re-extracted by water-chloroform. The aqueous fraction that remained from the petroleum ether-treated total extract was named "fraction A" and the aqueous fraction that remained from the chloroform-treated extract was called "fraction B". All extracts were lyophilized and kept at -70°C .

Phytochemical analysis

Phytochemical screening was carried out on the total extract of fig tree leaves for qualitative estimations, which were recorded as none [0], low [+], medium [++] and high [+++] levels, of alkaloids, saponins, tannins, flavonoids and glycosides (Shariat, 1989).

Experiments on animals

Fifty-five healthy male Wistar albino rats (body weight [BW]: 200-250 g) were purchased from the Razi Institute, Karaj, Iran and brought to the animal house at the Faculty of Veterinary Medicine, University of Tehran, Iran. They were housed at $21\pm 2^{\circ}\text{C}$, with a 12 h light/ dark cycle and allowed to have free access to food and water for seven days as an adaptation period.

Animals were divided randomly into 11 groups of five. All groups except group 11 (control group with normal diet) were fed with a hyperlipidemic diet that contained cholesterol (1%), cholic acid (0.1%) and olive oil (2.5%) for 12 days (Kimura *et al.*, 1997). Nine groups of animals were injected intraperitoneally (ip) daily for eight days with different doses of three extracts. Hydromethanolic (total) extract at 2.5, 5, 10 mg/kg BW, ip, and its two aqueous fractions, fraction A and fraction B, were used both at 10, 50, 250 mg/kg BW, ip, in the hyperlipidemic animals. Animals in the negative and positive control groups (with normal and hyperlipidemic diets, respectively) each received a normal saline ip injection alone (1 ml). After this period, all groups were fasted for 14 hours and then were anesthetized with chloroform, an inhalant anesthetic agent, using a desiccator. Intracardial blood samples were collected from each animal and, after abdominal sectioning, liver samples were collected. All liver and serum samples were kept at -70°C until

their analysis.

Measurement of total cholesterol

Lipid contents of liver samples were extracted by the method of Folch *et al.*, 1957. The TC levels in liver extracts and serum samples were measured by the method described by Fossati and Prencipe in 1982 with the use of the Cholesterol-SL Kit (ZiestChem Diagnostics, Tehran, Iran).

Statistical analysis

Data were expressed as mean averages \pm SD, and differences between means were analyzed by a one-way analysis of variance (ANOVA) and Tukey tests using Sigma Stat software (Systat Software Inc., Point Richmond, CA, USA). A p-value < 0.05 was considered to be significant.

Results

Phytochemical analysis

Phytochemical screening showed that the total extract of *Ficus carica* leaves had only a small amount of alkaloids [+], a moderate level of flavonoids [++], a large amount of tannins [+++], and no saponins [0] or glycosides [0].

Effects of extracts on cholesterol levels in the serum and liver

The effects of different doses of *Ficus carica* leaf extracts on total cholesterol levels (TC) in the serum and liver are shown in Figures 1 and 2. The TC in serum was significantly increased in the hyperlipidemic group of rats (1.40 ± 0.26 mmol/L) in comparison with the control group (1.01 ± 0.09 mmol/L, $p < 0.05$). The level of TC in the liver was significantly increased in the hyperlipidemic group (59.86 ± 10.35 mg/g) in comparison with control group (15.68 ± 2.06 mg/g, $p < 0.001$).

As shown in Figures 1 and 2, certain doses of *Ficus carica* leaf extracts lowered the level of TC significantly in both the serum and liver compared to hyperlipidemic groups (between $p < 0.05$ and $p < 0.001$). However, other doses had no significant effect at all or only decreased the level of TC in either the serum or the liver.

In hyperlipidemic rats, a dose of total extract of 5.0 or 10.0 mg/kg significantly lowered TC in the serum from 1.40 ± 0.26 mmol/L (untreated group) to 1.04 ± 0.18 and 1.07 ± 0.17 mmol/L, respectively. The levels of TC in the liver were lowered significantly by doses of total extract of 2.5, 5.0 and 10.0 mg/kg from 59.86 ± 10.35 mg/g (untreated) to 42.68 ± 2.88 , 34.50 ± 7.07 and 11.25 ± 7.58 mg/g, respectively.

All doses fraction A (10, 50 and 250 mg/kg) significantly lowered TC in the serum from 1.40 ± 0.26 mmol/L (untreated group) to 1.06 ± 0.14 , 1.04 ± 0.07 and

Figure 1: The effects of different doses of *Ficus carica* leaf extracts on total cholesterol (TC) levels in the serum of hyperlipidemic rats (Mean \pm SD; n=5 each group). C: control group with normal saline injection, ip; H: hyperlipidemic rats with normal saline injection, ip; T 2.5, T 5, and T 10: hyperlipidemic rats treated with total extract at 2.5, 5.0, and 10 mg/kg, ip, respectively; A 10, A 50, and A 250: hyperlipidemic rats treated with fraction A at 10, 50, and 250 mg/kg, ip, respectively; B 10, B 50, and B 250: hyperlipidemic rats treated with fraction B at 10, 50, and 250 mg/kg, ip, respectively. * Represents a significant difference ($p < 0.05$) between extract-treated groups when compared to the H group.

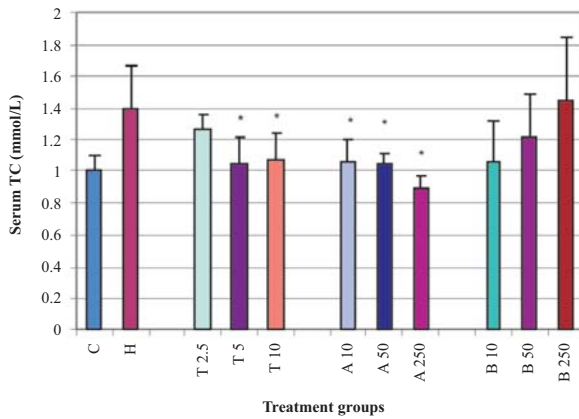
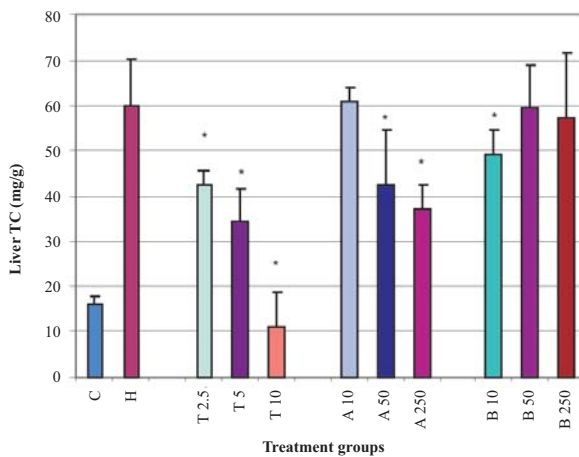


Figure 2: The effects of different doses of *Ficus carica* leaf extracts on total cholesterol (TC) levels in the livers of hyperlipidemic rats (mean \pm SD; n=5). C: control group with normal saline injection, ip; H: hyperlipidemic rats with normal saline injection, ip; T 2.5, T 5, and T 10: hyperlipidemic rats treated with total extract at 2.5, 5.0, and 10 mg/kg, ip, respectively; A 10, A 50, and A 250: hyperlipidemic rats treated with fraction A at 10, 50, and 250 mg/kg, ip, respectively; B 10, B 50, and B 250: hyperlipidemic rats treated with fraction B at 10, 50, and 250 mg/kg, ip, respectively. * Represents a significant difference ($p < 0.05$) between extract-treated groups and the H group.



0.90 \pm 0.08 mmol/L, respectively. The level of TC in the liver was lowered significantly by fraction A at 50 and 250 mg/kg from 59.86 \pm 10.35 mg/g (untreated) to 42.61 \pm 12.08 and 37.16 \pm 5.59 mg/g, respectively. Fraction B at dosages of 10, 50 and 250 mg/kg had conflicting effects on the level of serum TC without any

significant lowering effect at any dose. Although fraction B at 10 mg/kg lowered TC in the liver significantly (59.86 \pm 10.35 vs. 49.26 \pm 5.439 mg/g, $p < 0.05$), larger doses did not change it significantly.

Discussion

A number of medicinal plants and natural products are currently used in various traditional medical systems to treat hyperlipidemia. In rats treated with a hyperlipidemic diet in the present study, the cholesterol levels in both the serum and liver increased significantly in comparison with the control group that had a normal diet. In the treatment groups, one or more doses of different extracts of *Ficus carica* leaves in hyperlipidemic rats decreased the serum and liver cholesterol levels in a dose-dependent manner. The results of this study suggest that the effect of total extract on liver cholesterol was more pronounced than its effect on the serum cholesterol level. In addition, the lipid-lowering effect of total extract in the liver was also dose-dependent.

Fraction A caused a significant dose-dependent decrease in cholesterol levels in both the serum and liver. Since this fraction is aqueous-based and has shown consistently favorable results, it seems that fraction A is one of the best fractions that require more studies. Fraction B reduced cholesterol levels in the serum and liver to a lower extent, but the results were conflicting and not as considerable as the other extracts used in this study.

The findings of our study are in accordance with Canal *et al.* (2002), who reported that a chloroform extract obtained from a decoction of *Ficus carica* leaves had hypocholesterolemic effects in rats with streptozocin-induced diabetes.

The results of phytochemical screening tests showed that total extract had a moderate level of flavonoids and a large amount of tannins. In this regard, come data suggests that flavonoids, such as naringenin and hesperidin, could lower cholesterol levels significantly in both the plasma and liver (Cook and Samrnan, 1996; Bok *et al.*, 1999; Borradaile *et al.*, 2003; Lee *et al.*, 2003). Moreover, it has been showed that naringenin and its analogs could inhibit hepatic 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase activity and modulate plasma and hepatic lipids in rats fed with high levels of cholesterol (Lee *et al.*, 2003). Bok *et al.* (1999) reported that the hepatic activities of HMG-CoA reductase and acyl CoA cholesterol O-Acyl transferase (ACAT) were lower in rats that were fed with citrus peel extra or a mixture of citrus bioflavonoids (naringenin and hesperidin). Although the type of flavonoids and the mechanism(s) of action in different leaf extracts of *Ficus carica* on cholesterol levels are not known, one or more constituents of the extracts may play a role in the

cholesterol synthesis or metabolic pathways.

On the other hand, a report by Park *et al.*, (2002) showed that tannins had hypolipidemic effects when tannic acid supplemented rat feed for three weeks. They noted that tannic acid lowered both plasma lipid concentrations (cholesterol and triglyceride) and hepatic HMG-CoA reductase activity. Therefore, it can be suggested that inhibition of HMG-CoA reductase and ACAT enzyme activities may be involved in the cholesterol-lowering effect of the extracts used in this study.

In conclusion, *Ficus carica* leaf extracts were shown to lower the cholesterol levels within the liver and serum in rats with experimentally-induced nutritional hyperlipidemia. Further studies are now needed to explore the precise ingredient(s) and the mechanism(s) that are involved.

References

- Asadi, F.; Pourkabir, M.; Maclaren, R. and Shahriari, A. (2006) Alteration to lipid parameters in response to fig tree (*Ficus carica*) leaf extract in chicken liver slices. *Turk. J. Vet. Ani. Sci.* 30: 315-318.
- Bok, S.H.; Lee, S.H.; Park, Y.B.; Bae, K.H.; Son, K.H.; Jeong, T.S. and Choi, M.S. (1999) Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA and cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. *J. Nutri.* 129: 1182-5.
- Borradaile, N.M.; DeDreeu, L.E.; Barrett, P.H.; Behrsin C.D. and Huff, M.W. (2003) Hepatocyte apoB-containing lipoprotein secretion is decreased by the grape fruit flavonoid, naringenin via inhibition of MTP-mediated microsomal triglyceride accumulation. *Biochem.* 42: 1283-91.
- Campillo, J.E.; Torres, M.D.; Dominguez, E.; Romero, A. and Perez, C. (1994) *Ficus carica* leaf administration reduces hypertriglyceridemia in streptozocin diabetic rats. *Diabetologia.* 37 (suppl. 1): A213.
- Canal, J.R.; Torres, M.D.; Romero, A. and Perez, C. (2002) A chloroform extract obtained from a decoction of *Ficus carica* leaves improves the cholesterolaemia of rats with streptozocin-induced diabetes. *Acta. Physiol. Hung.* 87: 71-76.
- Cignarella, A.; Nastasi, M.; Caralli, E. and Puglisi, L. (1996) Novel lipid-lowering properties of *Vaccinium myrtillus* L. leaves, a traditional antidiabetic treatment in several model of rats dyslipidaemia: A comparison with ciprofibrate. *Thromb. Res.* 84: 311-22.
- Cook, N.C.; Samrnan, S. (1996) Flavonoids: chemistry, metabolism, cardioprotective effect and dietary sources. *J. Nut. Biochem.* 7: 66-76.
- De-Amorin, A.H.; Borba, H.R.; Carauta, L.D. and Kaplan, M.A. (1999) Anthelmintic activity of the latex of *Ficus carica*. *J. Ethnopharm.* 64: 255-258.
- Evans, W.C. (2002) *Trease and Evan's Pharmacognosy.* 15th edition, Saunders Company. pp: 214-248.
- Folch, J.; Lees, M. and Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497-509.
- Fossati, P.; Prencipe, L. (1982) Determination of cholesterol. *Clin. Chem.* 28: 2077.
- Galati, E.M.; Monforte, M.T.; Forestieri, A.M.; Miceli, N.; Bader, A. and Trorato, A. (1999) *Salvadora persica* L: hypolipidemic activity on experimental hypercholesterolemia in rat. *Phytomedicine.* 6: 181-5.
- Kimura, M.; Kurose, I.; Russell, J. and Granger, O.N. (1997) Effects of fluvastatin on leukocyte-endothelial cell adhesion in hypocholesterolemic rats. *Arterioscler. Thromb. Vasc. Biol.* 17: 1521-26.
- Lee, M.K.; Moon, S.S.; Lee, S.E.; Bok, S.H.; Jeong, T.S.; Park, Y.B. and Choi, M.S. (2003) Naringenin, 7-O-cetyl ether as inhibitor of HMG-Co reductase and modulator of plasma and hepatic lipids in high cholesterol-fed rats. *Bioorg. Med. Chem.* 11: 393-8.
- Nakamara, Y.; Susumu, I. and Yasuhide, T. (2000) Effects of Quercetin and Rutin on serum and hepatic lipid concentration feed steroid extraction and serum antioxidant properties. *J. Health. Sci.* 46: 229-40.
- Park, S.Y.; Bok, S.H.; Jeon, S.M.; Park, Y.B.; Lee, S.J.; Jeon, T.S. and Choi, M.S. (2002) Effect of rutin and tannic acid supplements on cholesterol metabolism in rats. *Nutrition Research.* 22: 283-295.
- Perez, C.; Canal, J.R.; Campillo, J.E.; Romero, A. and Torres, M.D. (1999) Hypotriglyceridemic activity of *Ficus carica* leaves in experimental hypertriglyceridemic rats. *Phyto. Res.* 13: 188-191.
- Perez, C.; Canal, J.R. and Torres, M.D. (2003) Experimental diabetes treated with *Ficus carica*: effect on oxidative stress parameters. *Acta Diabetol.* 40: 3-8.
- Rubnov, S.Y.; Kashman, R.; Rabinowitz, M.; Schlesinger, M. and McChoulam, R. (2000) Suppressor of cancer cell proliferation from fig (*Ficus Carica*) resin isolation and structure elucidation. *J. Nut. Prod.* 64: 993-6.
- Serraclara, A.F.; Hawkins, C.; Perez, C.; Dominguez, E.; Campillo, J.E. and Torres, M.D. (1998) Hypoglycemic action of an oral fig-leaf decoction in type-1 diabetic patients. *Diab. Res. Clin. Pra.* 39: 19-22.
- Shariat, S. (1989) *Analysis and recognition of planta medica: microscopic and chromatographic methods.* Mash-al Publication, Isfahan, Iran: 1-31, 101-164.
- Yansi, S.; Imaizumi, K. (1991) Effect of Indonesian medicinal plants, *Curcuma Xanthorrhiza* Roxb, on the level of serum glucose and Triglyceride, fatty acid desaturation, bile acid excretion streptozocin-induced diabetic rats [Abstract]. *Agr. Biol. Chem.* 55 (suppl. 12): 3005-3010.