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Original Article

Antidiabetic Effect of Hydroalcholic *Urtica dioica* Leaf Extract in Male Rats with Fructose-Induced Insulin Resistance

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Abstract

Background: *Urtica dioica* has been used as antihypertensive, antihyperlipidemic and antidiabetic herbal medicine. The purpose of this study was to study the effect of hydroalcoholic extract of *Urtica dioica* on fructose-induced insulin resistance rats.

Methods: Forty male Wistar rats were randomly divided into five groups including control, fructose, extract 50, extract 100 and extract 200. The control rat received vehicle, the fructose and extract groups received fructose 10% for eight weeks. The extract groups received single daily injection of vehicle, 50, 100 or 200 mg/kg/day for the two weeks. Blood glucose, insulin, last fasting insulin resistance index (FIRI), serum triglyceride (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), alanin trasaminase (AST) and alkaline phosphatase (ALP), leptin and LDL/HDL ratio were determined.

Results: Compared to control group, daily administration of fructose was associated with significant increase in FIRI, blood glucose and insulin, significant decrease in lepin, and no significant change in TG, HDL, LDL, LDL/HDL ratio, VLDL, ALT, and ALP. The extract significantly decreased serum glucose, insulin, LDL and leptin, and LDL/HDL ratio and FIRI. It also significantly increased serum TG, VLDL, and AST, but did not change serum ALP.

Conclusion: We suggest that *Urtica dioica* extract, by decreasing serum glucose, and FIRI, may be useful to improve type 2 diabetes mellitus. Also, by positive effect on lipid profile and by decreasing effect on leptin, it may improve metabolic syndrome.

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Keywords ● Fructose ● insulin resistance ● Urtica dioica

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Introduction

Diabetes mellitus occurs when the body can't use glucose normally, and is associated with increased serum triglycerides, decreased serum HDL and sometimes increased serum LDL.[1] According to ancient medical texts, *Urtica dioica* may be used for the treatment of high blood sugar.[2] Hypoglycemic activity of *Urtica dioica* was detected in a large pharmacological screen of European species with known potential anti-diabetic effects.[3] It has also been reported that the extract of the leaves or other parts of the plan were of benefits in conditions such as prostatic hyperplasia, Inflammation, arthritis rheumatoid, hypertension

and allergic rhinitis.[4] Urtica dioica has been reported to have histamine, formic acid, acetylcholine, acetic acid, butyric acid, leukotrienes, 5-hydroxytryptamine, and other irritants.[5,6] This study aimed to evaluate the effect of Urtica dioica leaf extract on blood glucose, lipid profile, insulin and leptin in rat model of fructose-induced insulin resistance.

Materials and Methods

Animal Maintenance

Forty male Wistar rats, weighting 200-250 g were obtained from the Animal Breeding Center, Jundishapur University of Medical Sciences, and were kept under standard conditions (12/12 light-dark cycle, 20-24°C, 55% humidity) with free access to water and food. All procedures were performed in accordance with the University guidelines for care and use of laboratory animals.

Plant Extraction

Urtica dioica was collected around the city of Ahwaz and identified by a faculty of the Department of Pharmacognosy, Jundishapur University of Medical Sciences. The leaves were dried under the shade and ground to powder by an electrical grinder. The extraction was prepared using maceration method. The powder was macerated for 72 hours at room temperature using 70% ethanol and 30% water. The mixture was filtered with Whatman filter paper (No 1), and the filtrate was centrifuged at 3000 rpm for 20 min. The supernatant was evaporated at ambient temperature and the extract powder (15.1% of leaf powder) was kept at 4˚C until used.[7]

Experimental Studies

One group of rats was assigned as sham group (n=8) and given tap water. Thirty two rats, given daily fresh fructose 10% in drinking water,[8] for eight weeks. Starting from the 6th week, they were randomly divided into four groups (n=8 each) including a control receiving intraperitoneal (IP) vehicle for *Urtica dioica*, and three other groups receiving single administrations of IP hydro-alcoholic extract of *Urtica dioica* at 50, 100 or 200 mg/kg.[9] Twenty four hours after the last IP injection, the animals were lightly anesthetized and blood samples were obtained by cardiac puncture.[10] The serum of blood samples were separated and were used to determine levels of blood glucose, insulin, fasting insulin resistance index (FIRI), leptin, triglycerides (TG), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol and hepatic enzymes.[5] Serum glucose levels were determined using glucose-oxidase method. The intra- and interassay variances were 2% and 4%, respectively. Fifty µl of serum was used for the measurement of insulin by immunoradiometric assay (Biosource INS-IRMA Kit). The intra- and interassay variances were 4% and 8%, respectively. Lipid profile, FIRI, alanin transaminase (ALT), and alkaline phosphatase (ALP) were determined by commercial kits and enzymic ways.[11]

Statistical Analysis

The data were expressed as mean±SEM. Data distribution was assessed by Shapiro-Wilk's test. The data were analyzed by one-way ANOVA and post hoc least significant different (LSD) tests. A P value of ≤ 0.05 was considered as significant.

Results

Effect of Fructose Administration

Compared to control group, daily administration of fructose for eight weeks was associated with significant increase in blood glucose (P<0.05), insulin (P<0.001), and FIRI (P<0.001) (figures 1-3).

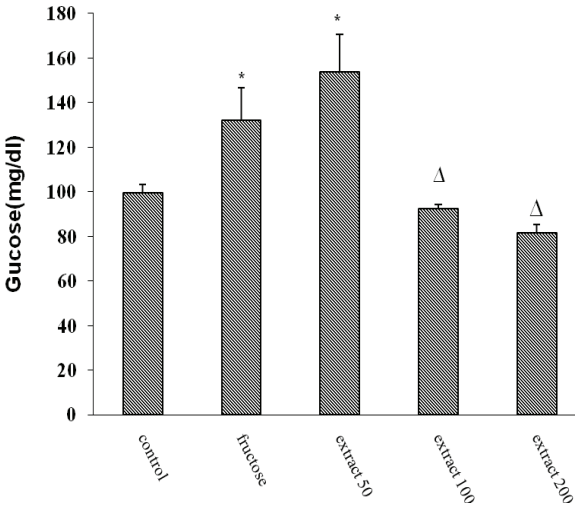

Figure 1: Serum glucose concentration (mean±SEM n=8 each) of control, fructose-treated and *Urtica dioica* extract-treated rats at 50, 100 or 200 mg/kg/day. *Indicates significant difference from the control group; ΔIndicates significant difference from fructose group

Effect of Urtica Dioica Extract

Compared to vehicle, *Urtica dioica* extract at 100 mg/kg (P<0.01) and 200 mg/kg (P<0.001) significantly decreased serum glucose (figure 1). Moreover, compared to the vehicle, *Urtica dioica* at 50, 100 and 200 mg/kg significantly (P<0.001) decreased serum insulin and FIRI (figures 2 and 3).

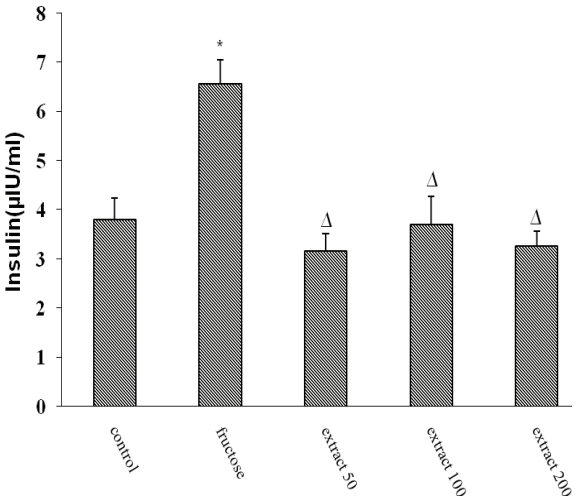

Figure 2: Serum insulin concentration (mean±SEM, n=8 each) of control, fructose-treated and *Urtica dioica* extract-treated rats at 50, 100 or 200 mg/kg/day). *Indicates significant difference from the control group; ΔIndicates significant difference from the fructose group

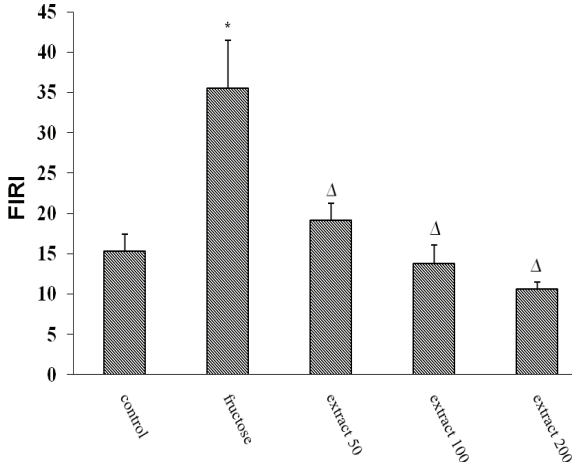

Figure 3: The values (mean±SEM, n=8 each) of fasting insulin resistance index (FIRI) of control, fructose-treated and *Urtica dioica* extract-treated (50, 100, 200 mg/kg/day) rats. *Indicates significant difference from the control group; ΔIndicates significant difference from fructose group

Effect of Urtica Dioica Extract on Lipid Profile

Daily administration of fructose for eight weeks did not change serum TG, total cholesterol, VLDL, LDL-cholesterol, HDL-cholesterol, LDL/HDL ratio compared to those of the control group (table 1).

Compared to the fructose group, *Urtica dioica* extract at 50 mg/kg significantly (P<0.05) increased serum TG and VLDL-cholesterol, and significantly (P<0.05) decreased serum LDL and LDL/HDL ratio (table 1). Moreover, compared to fructose group, the extract at 100 and 200 mg/kg/day significantly (P<0.05) increased TG, and significantly (P<0.05) decreased LDL and LDL/HDL ratio.

Effect of Extract on Hepatic Enzymes

Treatment with fructose did not change serum ALP or AST significantly relative to the control group. At none of the doses used *Urtica dioica* extract changed serum ALP relative to that of the fructose group. However, at 100 and 200 mg/kg/day, the extract increased serum AST relative to that of the fructose group (table 1).

Effect of Extract on Leptin

The fructose-treated group had a significantly (P<0.05) higher serum leptin compared to that of the control group. *Urtica dioica* extract at 50 and 100 mg/kg/day, but not 200 mg/kg/day, reduced serum leptin compared to that of the fructose group (table 1).

Discussion

Type 2 diabetes is a multi-factorial disease, frequently associated with a cluster of pathologies including obesity, hypertriglyceridemia, impaired glucose tolerance, and insulin resistance. Fructose intake may be associated with increased risk of type 2 diabetes through several biological mechanisms.[12] A higher fructose intake may play a role in

Table 1: The values (mean ±SEM, n=8 each) of serum lipid profile, hepatic enzymes, and leptin of control, fructose-treated and *Urtica dioica* extract-treated (50, 100, 200 mg/kg/day) rats

Groups	Control	Fructose	Extract (50 mg/kg)	Extract (100 mg/kg)	Extract (200 mg/kg)
Total cholesterol (mg/dl)	114.4±15	97.6±21	81.2±8.4	103.83±6	110.17±14
TG (mg/dl)	83.4±8	85±3	134.4±32$^{\Delta}$	108.3±10$^{\Delta}$	104.67±10$^{\Delta}$
HDL-cholesterol (mg/dl)	52±3.8	38.8±6.7	32.2±5.3	47.5±4.1	51.17±5.9$^{\Delta}$
LDL (mg/dl)	48.4±11	41.8±11	20.36±4.6$^{\Delta}$	34.67±3.7 $^{\Delta}$	38.07±7.05$^{\Delta}$
VLDL (mg/dl)	16.68±1.8	17±0.62	26.88±6$^{\Delta}$	21.67±2.1	20.93±2.1
LDL/HDL	0.9 0±0.15	0.98±0.15	0.6±0.07$^{\Delta}$	0.75±0.1$^{\Delta}$	0.73±0.09$^{\Delta}$
ALT	41.2±3.6	40.6±2.7	34±5.6	47.8±4.9$^{\Delta}$	53.5±4.5$^{\Delta}$
ALP	304.4±48	297.4±28.2	279.2±29	292.6±37	299.8±58
Leptin (ng/ml)	1.34±0.09	1.6±0.03*	1.14±0.02$^{\Delta}$	0.6±0.2$^{\Delta}$	0.95±0.17

TG: triglyceride; HDL: high density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; ALT: alanine transaminase; ALP: alkaline phosphatase; *Indicate significant difference (P≤0.05) from the control group; $^{\Delta}$Indicates significant difference from the fructose group

an increase in body weight due to the positive energy balance. Positive energy balance leads to obesity that is associated with a higher concentration of nonesterified fatty acids, which may reduce insulin sensitivity, increase hepatic glucose production, and have a deleterious effect on the beta cell function.[13] Golalipour et al showed that the protective administration of hydroalcholic extract of *Urtica dioica* had hypoglycemic effect as well as protective activity on pancreatic beta cells in hyperglycemic rats.[14] Our findings are agreement with those of Ahangarpour,[11] and Jalal's,[15] studies that higher intake of fructose increased glucose, insulin, and FIRI. Therefore fructose may increase the risk of type 2 diabetes. The increase of serum glucose by fructose in our study is similar to that of Magno et al.[16] who showed that glucose concentrations increased to 145-150 mg/dl in animals drinking 10% fructose solutions. This shows that animals in the present study were diabetic. *Urtica dioica* is known in Iran's folk medicine to have hypotensive and antidiabetic activities.[4] Domola et al showed that *Urtica dioica* reduced blood glucose levels upon oral ingestion.[17] Moreover, it was shown that a preparation containing various plants with *Urtica dioica* extract had antidiabetic activity.[18] However, other studies reported no hypoglycemic action of this plant.[19] The results of this study showed that hydroalcoholic extract of *Urtica dioica* leaves could decrease the blood glucose and insulin in hyperglycemic rats, which may be caused in part by the reduction of insulin resistance. Cholesterol is one of the body fats and is an important building block in the structure of biological membranes, and used in the biosynthesis of steroid hormones, bile acids and vitamin D. Moreover, the high cholesterol concentration in the blood increases the risk of developing atherosclerosis and related cardiovascular diseases.[20] Low-density lipoprotein takes the cholesterol from liver to tissues, whereas high-density lipoprotein facilitates the translocation of cholesterol from the peripheral tissues to liver for catabolism. Therefore, HDL has a useful effect in reducing serum cholesterol and the increase of its level in serum is suggested.[21] The LDL/HDL ratio is an important predictor of coronary heart disease risk. Low dose of *Urtica dioica* decreased LDL/HDL cholesterol ratio in comparisons with fructose group. This finding is similar to that of a previous finding by Daher et al.[22] In this study *Urtica dioica* extract decreased leptin compare to the fructose group. Leptin secretion by adipocytes is stimulated by insulin, and plasma leptin significantly correlates with plasma insulin.[23] Thus the decreasing effect of *Urtica dioica* on plasma insulin level may play a role in leptin reduction. Leptin stimulates vascular inflammation, oxidative stress, and vascular smooth muscle hypertrophy that may contribute to the pathogenesis of type 2 diabetes mellitus, hypertension, atherosclerosis, and coronary heart disease. By decreasing serum leptin *Urtica dioica* extract can improve these diseases.[24] Alkaline phosphatase and ALT are enzymes found in the highest amounts in the liver. They leak into the blood, when parenchymal liver cells are damaged, resulting in elevated levels of these enzymes in the bloodstream, however, some patients with liver damage have normal or near normal ALT.[25] Serum levels of ALT and ALP show that no liver damage had occurred during in the present study, which show that that low dose of the extract decreased ALT significantly and showed a tendency to decrease ALP. Therefore, this dose of extract had more efficacies to decrease liver damage.

Conclusion

This study demonstrated that *Urtica* dioica extract had hypoglycemic and antidiabetic activities with no deleterious effect on hepatic enzymes.

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Conflict of Interest: None declared.

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