

Hypoglycemic effects of three Iranian edible plants; jujube, barberry and saffron: Correlation with serum adiponectin level

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Abstract: One of the most common disorders of the endocrine system is diabetes mellitus. This disease is associated with dyslipidemia. Adiponectin is a protein hormone that secreted by adipocytes and has an important role in regulating of glucose and fatty acid metabolic pathways. This study was designed to investigate the changes in serum level of adiponectin in diabetic rats treated with hydroalcoholic extracts of three medicinal plants; jujube (*Ziziphus jujuba*), barberry (*Berberis vulgaris*) and saffron (*Crocus sativus*) in comparison with quercetin. Streptozotocin -induced diabetic male rats were gavaged with specified doses of the extracts (25 and 100mg/kg) for two weeks. At the end of treatment period, fasting blood specimens were collected. The levels of adiponectin, fasting blood sugar (FBS), total Cholesterol, triglycerides, HDL-C and LDL-C were measured. Statistical analysis showed that serum levels of triglyceride and VLDL decreased significantly ($P<0.05$) in all treated groups. FBS level in all treated groups, decreased significantly and reach to normoglycemic level ($P<0.05$). Except Jujube, other plant extracts had no effect on cholesterol. Jujube in two doses (25 and 100mg/kg) could increased significantly HDL-C ($P<0.05$) with no effect on total cholesterol and LDL-C. Serum adiponectin level increased in all treated groups. These beneficial effects of *C. sativus*, *B. vulgaris* and *Z. jujube* extracts and quercetin in diabetic rats may be associated with increase in adiponectin level.

Keywords: Adiponectin, dyslipidemia, *Ziziphus jujube*, *Berberis vulgaris*, *Crocus sativus*.

INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine disorders, which characterized by hyperglycemia and abnormal metabolism of carbohydrates, lipids and proteins. Plasma adiponectin level is strongly associated with the risk of developing DM (Dalamaga *et al.*, 2012).

Adiponectin is a specific protein, secreted by adipose tissue, which its secretion decreases during the early stages of obesity or insulin resistance (Skvarca *et al.*, 2012). Studies showed that the molecular mechanism of adiponectin to improve insulin sensitivity is related to activity of AMPK (AMP-Activated Protein Kinase) enzyme, which increases glucose absorption in muscles. This enzyme reduces the plasma glucose level through lowering the gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, in liver. Adiponectin causes phosphorylation of acetyl coenzyme A carboxylase, and controls lipid synthesis by stimulating AMPK. Also, it increases expression of genes involved in fatty acid oxidation. This hormone lowers the free fatty acids in muscles through their oxidation, which leads to a decrease in muscle triglyceride content (Gable *et al.*, 2006 and Gil-campos *et al.* 2004).

Southern Khorasan Province is located in neighboring Afghanistan in eastern part of Iran. This region has a dry climate with significant difference between day and night temperatures. According to its climate characteristics, Southern Khorasan has its specific local plants, including saffron, jujube, and barberry which widely used in the food and pharmaceutical applications. Saffron (*C. sativus*) can decrease blood levels of lipids and cholesterol, as well as blood pressure due to possess active compounds, such as crocetin, crocin, carotenoids (e.g., Beta-carotene, lycopene and xanthin), and vitamins (e.g., Riboflavin and thiamine). It is traditionally used for treatment of disorders such as DM (Sheng *et al.* 2006).

Jujube (*Z. Jujuba*) contains about 5% protein, 4% carbohydrate, and a considerable amount of vitamin C and minerals. There are ziziphic and zizimauritic acids in aqueous extract of its wooden parts. Jujube causes a decrease in the blood levels of glucose and lipids, and it has been reported to make a significant decline in triglyceride, LDL and cholesterol levels (Shirdel *et al.*, 2009).

Barberry (*B. vulgaris*) is a traditional medicinal plant for treating type 2 diabetes. Berberine, oxyacanthine, and berbamine alkaloids are present in all parts of the plant. Its fruits contain 4% carbohydrate, malic acid, tartaric acid, and some resin. Berberine can reduce blood sugar levels in diabetic patients. According to some studies in animal models, its consumption can decrease blood sugar

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and lipid levels and improve insulin function within the body (Gulfraz *et al.*, 2008 and Meliani *et al.*, 2011).

Volatile oils, alkaloids, flavonoids, tannins, vitamins, and plant acids are some of the important compounds of medicinal plants. Flavonoids are effective compounds, which are mostly found in higher plants. They detoxify different kinds of toxins within the body. Quercetin constitutes more than 60% of all flavonoids present in the human diet. Studies show quercetin decreases the serum glucose level, oxidative stresses, and blood vessel's damages in rats (Bakhshaeshi *et al.*, 2012 and Jeong *et al.*, 2012). Hypolipidemic activity of quercetin has been demonstrated in streptozotocin-induced diabetic rats (Vessal *et al.*, 2003).

To assess the hypoglycemic activity mechanism of *C. sativus*, *Z. Jujuba* and *B. vulgaris*, we consider the changes in serum adiponectin levels of streptozotocin-induced diabetic rats treated by the hydroalcoholic extracts of these plants.

MATERIALS AND METHODS

Preparation of hydroalcoholic extracts

Jujube and barberry fruits and saffron petals collected from Birjand, Southern Khorasan, Iran. Fresh plant materials were washed, air dried and then homogenized to fine powder and stored in airtight bottles. To prepare alcoholic extracts, plant powders were mixed with 80% ethanol (in 1:9 ratio) using heat-shaker for 24 hours and then filtered by grade 1 qualitative filter paper (Whatman, Sigma-Aldrich, USA). Alcohol evaporated in a rotary evaporator. Extracts dried at 37°C and were kept in amber stained bottles in a dry place until they were used. Quercetin dihydrate (Sigma-Aldrich, USA) was obtained as a commercial product and was administered to the rats in scheduled doses.

Animals and treatments

In this experimental study, 45 mature male Sprague-Dawley rats weighting 200 to 220g were obtained from Pasteur Institute (Tehran, Iran). To get adapted to the new environment, animals were kept for one week under controlled laboratory conditions (light regime of 12h: 12h light:dark cycle, temperature 22±3°C) with free access to food and water. Induction of diabetes in the rats was attempted using intraperitoneal injections of streptozotocin (60mg/kg Body Weight). One week later, blood glucose levels were measured and Animals with plasma levels above 300mg/dl were considered diabetic. Forty diabetic rats were randomly assigned to eight groups of five animals each and treated as following:

- Groups 1 and 2: Were given the hydroalcoholic extract of jujube at 25 and 100mg/kg body weight, respectively.

- Groups 3 and 4: received the same doses of saffron hydroalcoholic extract.
- Groups 5 and 6: hydroalcoholic extract of barberry was prescribed at 25 and 100mg/kg body weight, respectively.
- Group 7: received 15mg/kg quercetin intraperitoneally.
- Group 8 was diabetic control group, and received 0.9% saline.

Five healthy rats were designated as the control group, and received 0.9% saline.

Diabetic treatment groups were fed daily with the extracts by gavage syringe, at a fixed time. After completion of treatment (14 days), serum specimens were prepared from blood samples, taken from their hearts.

Measurement of FBS and other biochemical factors

Serum concentrations of FBS, triglyceride, cholesterol, LDL, and HDL were measured by photometric methods, using appropriate diagnostic kits (Pars Azmun, Iran).

Measurement of adiponectin

Serum adiponectin level was quantitatively measured by ELISA using rat adiponectin ELISA kits (Glory Science, Taiwan). In each group, tests were performed in duplicate. The experimental protocol was approved by the ethics committee of Birjand University of Medical Sciences.

STATISTICAL ANALYSIS

Statistical analysis of data was performed using One-Way ANOVA (Tukey's test). The significance of differences (mean levels of glucose, adiponectin, etc.) between the control and treated groups were evaluated using Student's t-test. P-values of 0.05 or less were considered significant. SPSS version 16 software (SPSS Inc., Chicago, IL, USA) and Microsoft Excel were employed for performing statistical tests and drawing graphs, respectively.

RESULTS

FBS

The FBS concentrations of all groups of rats during experimental period are shown in fig. 1. There was a significant difference ($P<0.05$) between all treated groups and diabetic group.

Lipid profile

The effect of treatment with barberry, saffron, and jujube extracts and quercetin on biochemical factors in STZ-induced diabetic rats is reported in table 1. The blood triglyceride and VLDL levels of diabetic rats were significantly higher than those in normal rats. A significant decrease ($P<0.05$) in blood triglyceride and VLDL levels were observed in the diabetic groups treated with jujube, saffron, barberry and quercetin. There are no significant differences in Cholesterol, HDL and LDL of

normal and diabetic rats. But jujube-treated groups of diabetic rats (25 and 100mg/kg) had a statistically significant increasing in HDL level ($P<0.05$) compared with the normal group.

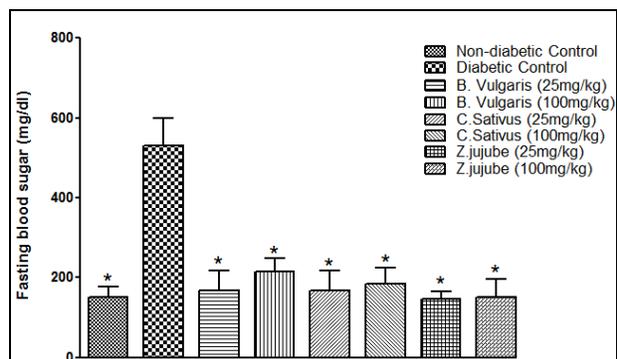


Fig. 1: The effect of hydroalcoholic extract of barberry, saffron and jujube on FBS level (mg/dl). (Mean±SD) *Significant differences with diabetic control group

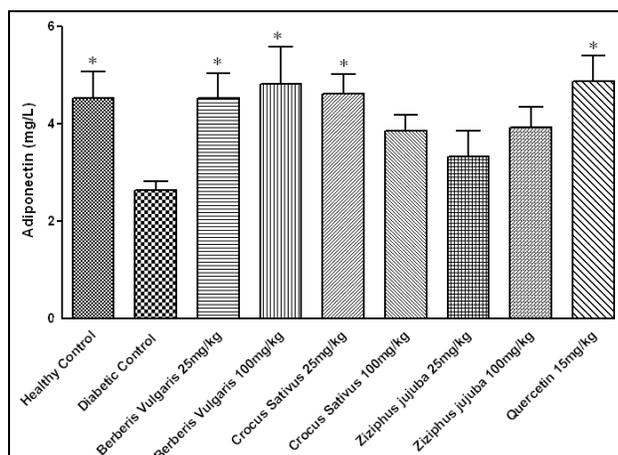


Fig. 2: The effect of hydroalcoholic extract of barberry, saffron and jujube on adiponectin level (mg/L). (Mean±SD) *Significant differences with diabetic control group

Adiponectin level

Serum adiponectin was significantly lower in the diabetic rats than in the normal rats ($P<0.05$). After two weeks of quercetin and plants extracts treatment, adiponectin levels were increased in all treated groups. However, there were statistically significant differences ($P<0.05$) of serum adiponectin levels between non-treated diabetic rats and rats treated by barberry (25 and 100mg/kg), saffron (25 mg/kg) and quercetin (15mg/kg) (fig. 2).

DISCUSSION

The results of this study indicate that *C. sativus*, *B. vulgaris*, *Z. Jujuba* and quercetin dihydrate may improve serum glucose level of diabetic rats by increasing of adiponectin levels of their blood. In diabetic rats, which were treated with quercetin, a significant decrease

($P<0.05$) in serum triglycerides besides increased adiponectin levels was seen. Also, by oral prescription of hydroalcoholic extracts of *C. sativus* and *B. vulgaris* to diabetic rats, their serum adiponectin levels increased significantly. Considering the results of similar studies, it could be concluded that adiponectin has a reverse relationship with glucose, triglyceride, VLDL, and cholesterol, and a direct relationship with HDL (Qiao *et al.*, 2008). Diabetes type 1 is associated with low levels of adiponectin. Since the increase of adiponectin is accompanied by decrease of diabetes-related factors such as glucose and triglyceride, a direct relationship between adiponectin and insulin could be inferred (Berg *et al.*, 2001 and Weyer *et al.*, 2001). As mentioned earlier, the molecular mechanism of adiponectin in increasing insulin susceptibility is apparently related to AMPK enzyme activity, which intensifies glucose absorption in muscles and controls lipid synthesis, eventually (Scvarca *et al.*, 2012). Moreover, Adiponectin increases the expression of genes that are involved in fatty acid oxidation (Yamauchi *et al.*, 2002). In this case, adiponectin regulates glucose and lipid metabolism through phosphorylation and activation of the 5'-AMP-activated protein kinase. Furthermore, adiponectin has beneficial effects on metabolic disorders due to stimulation of fatty acid oxidation in muscle cells by the sequential activation of AMPK, p38MAPK and PPAR alpha leading to a tissue decrease content of triglycerides in the liver and skeletal muscle, and improving insulin sensitivity *in vivo* (Yamauchi *et al.*, 2001 and Yoon *et al.*, 2006). In the present study, *C. sativus*, *B. vulgaris* and *Z. Jujuba* extracts decreased serum triglyceride levels considerably, and subsequently VLDL synthase, similar to the actions of quercetin. Significant decrease in serum triglycerides is consistent with researches conducted by Sheng *et al.*, (2006), Shirdel *et al.*, (2009) and Shidfar *et al.*, (2012). The hydroalcoholic extracts did not affect total cholesterol and LDL levels. Although in some previous researches in this field, reduction of total cholesterol and LDL levels, have been observed (Ashraf *et al.*, 2013 and Chen *et al.*, 2008 and Shirdel *et al.*, 2009).

Given the inverse relationship between plasma HDL and triglyceride concentrations, by reduction of plasma triglycerides levels in rats who received the plant extracts, HDL concentrations must be increased. But only *Z. Jujuba* extract could cause a significant increase in HDL levels, which is consistent with Shirdel *et al.* results (Shirdel *et al.*, 2009). Many cellular stresses such as increased glucose concentrations cause a rise in formation of free radicals (ROS). Because of low levels of antioxidant enzyme expressions, pancreatic beta-cells are vulnerable to oxidative stress, which can lead to chronic inflammation and insulin resistance. Studies have shown that natural antioxidants lower the risk of chronic diseases (such as diabetes and fatty liver) and improve the health state (Vassort and Turan., 2010). Jujube contains natural

Table 1: The effects of barberry, saffron and jujube extracts and quercetin on biochemical factors in STZ-induced diabetic rats

Experimental groups	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Diabetic control	162±44.3 ^a	87±8.1 ^a	33.8±7 ^a	21.1±8.7 ^a	32±8.7 ^a
Healthy control	43.5±10.4 ^b	64.4±6.7 ^a	45.3±5.3 ^a	23.7±11.8 ^a	8.7±2 ^b
Diabetic+ <i>Berberis vulgaris</i> (100mg/kg)	60.1±14.5 ^b	74.5±4.2 ^a	33.8±9.4 ^a	28.6±12.2 ^a	12±2.9 ^b
Diabetic+ <i>Berberis vulgaris</i> (25mg/kg)	57.6±23.5 ^b	66.1±8.1 ^a	30±4.6 ^a	24.5±7.4 ^a	11.5±4 ^b
Diabetic+ <i>Crocus sativus</i> (100mg/kg)	29.7±10 ^b	51.8±9.6 ^a	29.2±7.1 ^a	16.6±7.4 ^a	5.9±2 ^b
Diabetic+ <i>Crocus sativus</i> (25mg/kg)	41.2±18.3 ^b	61.4±11 ^a	26.2±11.6 ^a	26.9±5.7 ^a	8.2±3.6 ^b
Diabetic+ <i>Ziziphus jujubamil</i> (100mg/kg)	21.6±6.8 ^b	78.4±20.5 ^a	53.4±4.7 ^b	20.6±15.5 ^a	4.3±1.3 ^b
Diabetic+ <i>Ziziphus jujubamil</i> (25mg/kg)	38.8±33.5 ^b	80.5±12 ^a	54.4±7.5 ^b	21±12.9 ^a	5.1±2.2 ^b
Diabetic+quercetin (15 mg/kg)	39.53±3.3 ^b	77.63±21.7 ^a	35.09±4.4 ^a	22.3±13.8 ^a	7.9±0.6 ^b

Data are presented as the Mean ±SD for five rats in each group. In each column, significant differences (P<.05) with the diabetic control group are shown by different letters

antioxidants, such as vitamin C, carotenoids and flavonoids (Sharif *et al.*, 2009). Barberry has antioxidative properties due to ingredients like berberine, saponin, flavonoids, alkaloids, steroids, and high levels of vitamin C (Aryane *et al.*, 2007 and Sabir *et al.*, 1978). According to Gulfranz *et al.* (2008), barberry extract stimulates insulin secretion from pancreatic beta cells, thus reduces blood sugar. Berberine can decrease blood sugar by mechanisms such as activating protein kinase B enzyme, increasing glucose uptake through AMPK-p38 MAPK pathway, increasing insulin susceptibility, and stimulating glucose uptake by insulin (Yin *et al.*, 2008). Active ingredients of saffron such as crocin, possess hypoglycemic effects (Sheng *et al.*, 2006). Crocin could selectively inhibit pancreatic lipase which indirectly leading to malabsorption of fat. Adiponectin as an antioxidant has reverse correlation with oxidative stress, inflammation and chronic diseases such as diabetes (Chen *et al.*, 2012). The results of this study indicate that oral administration of *C. sativus*, *B. vulgaris*, *Z. Jujuba* and quercetin can decrease blood sugar to the normoglycemic level with beneficial effects on lipid metabolism in the body. This, maybe through activation of adiponectin signaling pathways, that more investigation is needed.

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