TÜBİTAK

Original Article

Turk J Med Sci
2011; 41 (4): 685-691
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1004-802

Anti-diabetic effects of an alcoholic extract of *Juglans regia* in an animal model

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Aim: Many traditional treatments have been recommended in the alternative system of medicine for treatment of diabetes mellitus. *Juglans regia* is one of the medicinal plants used in traditional Iranian medicine as a treatment for diabetes, but little scientific documentation supports its antidiabetic action. The purpose of this study was to investigate antidiabetic effect of *J. regia* leaves in type 1 diabetes induced animal model.

Materials and methods: Four groups of animals were selected. Group I animals were fed a normal diet. Animals of groups II, III, and IV received streptozotocin. Animals of groups III and IV were treated with *J. regia* leaf extract 200 and 400 mg/kg body weight for 28 days. The various parameters studied included body weight, blood glucose, triglycerides, cholesterols, LDL, VLDL, HDL, insulin, and glycated hemoglobin in all groups.

Results: Treatment with the *J. regia* extracts resulted in a significant decrease in blood glucose, glycosylated hemoglobin, LDL, triglyceride, and total cholesterol, and a significant increase in insulin and HDL level.

Conclusion: The plant extract is capable of ameliorating hyperglycemia in streptozotocin-induced diabetic rats and is a potential source for active agent(s) for diabetes mellitus. It is concluded that *J. regia* leaf extract can be potentially used to control type 1 diabetes.

Key words: *Juglans regia*, streptozotocin, insulin, diabetes mellitus, glucose, triglyceride, cholesterol

Introduction

Diabetes mellitus is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, action, or both. It has already been established that chronic hyperglycemia resulting from diabetes is associated with long term damage, dysfunction, and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels (1). The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million people likely to be diabetic by the year 2030 compared to an estimated 191 million sufferers in 2000 (2). Herbal drugs are gaining popularity in the treatment of diabetic mellitus (3). The major advantages of herbal medications seem to be their efficacy, low incidence of side effects, and low cost. A review of the literature reveals that 15%-20% of diabetic patients are suffering from insulin-dependent diabetes mellitus (IDDM), also known as type-I diabetes (4). This condition, IDDM, is noted both in adults and children (4,5). It is characterized by an elevation of both fasting and postprandial blood sugar levels. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting

Received: 28.04.2010 – Accepted: 13.07.2010

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the eyes, kidneys, nerves, and arteries (6). These may be delayed, lessened, or prevented by maintaining blood glucose levels that are close to normal.

In modern medicine, no satisfactory effective therapy is yet available to cure diabetes mellitus. Insulin therapy is used for the management of diabetes mellitus, but there are several drawbacks such as insulin resistance, anorexia nervosa, brain atrophy, and fatty liver after chronic treatment (7,8). Besides the use of insulin for the treatment of insulin dependent diabetes mellitus (IDDM), other approaches for the control of hyperglycemia include the use of amylin analogues, which regulate gastric emptying, and intestinal alpha-glucosidases inhibitors such as acarbose, miglitol, and voglibose, which delay postprandial hyperglycemia.

Recently, there has been increasing interest in the use of medicinal plants. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used throughout the world to prevent or cure diseases, scientific evidence of the efficacy of these plants is lacking in most cases. Today, however, it is necessary to provide scientific proof in order to justify the use of a plant or its active components (9). At this time, approximately 1200 types of therapeutic plants are thought to reduce glucose level in diabetes (10). The search for suitable anti-hyperglycemic agents has focused on plants used in traditional medicine because of indications that natural products may provide better treatment than the drugs that are currently being used (11).

The preliminary phytochemical studies of an ethanol extract of *Juglans regia* showed the presence of alkaloids, flavonoids, and saponins (12). Because any of these phytoconstituents may be responsible for the extract's pharmacological activity, more detailed studies of the phytoconstituents should be undertaken in order to confirm the exact mode of the pharmacological action**.** The purpose of this study was merely to raise awareness about the anti-diabetic activity of the alcohol extract *Juglans regia (J. regia)*.

Materials and methods

Plant material and extraction procedure

The *J. regia* leaves were collected from a garden in the east of the city of Yasouj. The fourth and fifth leaves from the apex of healthy plants were plucked, washed thoroughly under running tap water, shade dried for 5 days, and ground to a fine powder in an electric mixer. The powdered plant material (700 g) was twice subjected to extraction with 90% ethanol at room temperature, with each extraction lasting 24 h. The extract was filtered using Whatman No 1 filter paper. The filtrate was evaporated using a Soxhlet evaporator until dry and 80.4 g of the extracted powder were obtained. The dried powder was diluted with distilled water to concentrations of 200 and 400 mg/kg. Our experiments involved Wistar rats which were procured from the animal house of the department of physiology. The Institutional Animal Ethics Committee (Yasouj University of Medical Sciences' local committee on ethics) approved the experimental protocols in the present study (88.11.17.12.). The animals were maintained under standard conditions, in temperatures of 20 ± 5 °C with a regular 12 h light/dark cycle. They were allowed free access to standard laboratory food and water ad libitum throughout the experiment. At 6 weeks of age, the animals, all of which had a body weight ranging between 160 and 220 g, were distributed into 4 groups, irrespective of sex. Each group consisted of 8 animals and the groups were divided as follows: a control group (Group I), a diabetic group (Group II), a diabetic group treated with daily dosages of 200 mg/kg of the extract taken from the *J. regia* leaves (Group III), and a diabetic group treated with daily dosages of 400 mg/kg of the same extract (Group IV).

Experimental design

Animals in Groups II, III, and IV were rendered diabetic by single intraperitoneal (IP) injection of streptozotocin (STZ - 55 mg/kg) (Sigma, St. Louis, MO, USA) freshly prepared in 0.1 mol of citrate buffer (pH 4.5). Group I animals were injected with only the buffer. Blood was drawn from the tails of conscious rats 72 h after they were given the injections and glucose was estimated by glucometer. Diabetes was identified by glucose results; rats with a glucose level over 300 mg/dL were considered to be diabetic and were used in the experiment (13). Blood glucose was similarly monitored every week until autopsy. After 10 days of STZ injections, animals in Group III began receiving daily dosages of 200 mg/kg while Group IV received 400 mg/kg daily of the *J.*

regia leaf extract. The animals were given the extract orally by an intragastric tube once daily for 28 days. The stock solution was prepared for multiple groups, such that 1 mL of extraction was administered per day for each animal. Body weight was recorded weekly for each group. After 28 days of treatment, rats were fasted overnight and autopsied under light ether anesthesia. Blood was collected from the heart, transferred immediately into centrifuge tubes, and allowed to clot on order to obtain a serum.

Plasma glucose was estimated by Trinder's method using a GOD/POD kit (14). Total cholesterol was estimated by using Parekh and Jung's method (15). Glycosylated hemoglobin was determined according to the ion-exchange resin method (16). Triglycerides were measured with the enzyme-colorimetric method (17). HDL-cholesterol was assayed by the method of Burstein et al. (18). LDL-cholesterol and VLDL-cholesterol were measured by using the formula of Friendwald et al. (19).

Statistical analysis

Results were analyzed statistically using an analysis of variance (ANOVA) and represented as mean ± standard error (SE). Wherever the variance value was found to be significant (5%), Duncan's multiple range test (DMRT) was applied.

Results

The animals' final body weight showed a significant increase from initial body weight in Groups I and IV. There was a significant decrease in body weight compared to initial body weight in the diabetic group (Table 1). The failure of the diabetic rats to gain weight during the 4 week period corresponded with the hyperglycemia seen during this period. Animals in Group III and IV demonstrated greater weight gain than the diabetic group (Group II), but less than that of the control group (Group I).

Figure 1 shows changes in the rats' fasting blood glucose levels over the course of 28 days. Control rats did not show any significant variation in blood glucose throughout the experimental period. Administration of STZ (55 mg/kg) led to over fourfold elevation of blood glucose levels which remained consistent over the 28-day period. At a potency of 200 mg/kg, the *J. regia* extract reduced the hyperglycemia significantly compared to diabetic group but failed to restore the level to that of control group, whereas the treatment using 400 mg/kg almost reached the same level as the control group ($P < 0.05$).

Table 1. Effects of *Juglans regia* leaves extract on body weight levels (g).

Group	Initial	Final
Control I	175.4 ± 2.76[b]	208.4 ± 0.81[d]
Diabetic II	172 ± 4.15[b]	143.2 ± 5.21[a]
STZ+200 III	175.5 ± 6.91[b]	180.1 ± 4.32[b]
STZ+400 IV	168.4 ± 3.49[b]	185 ± 6.44[b,c]

The values are mean ± SE for 8 rats in each group. Means with different superscript (a, b, c, d) within a column are significantly different from each other at $P < 0.05$ as determined by Duncan's multiple range test.

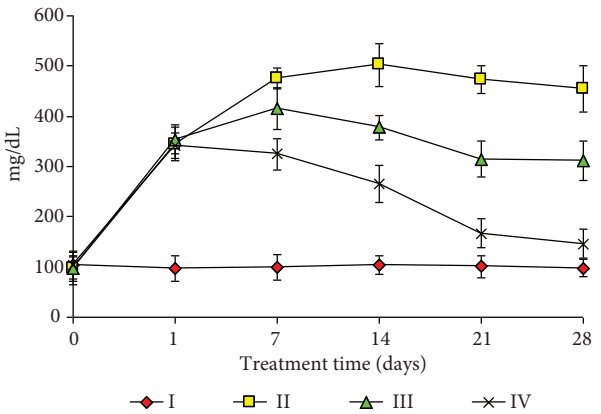

Figure 1. The effects of *J. regia* leaves extract on blood glucose levels are shown for a control group (I), a diabetic group (II), a diabetic group treated with a daily dosage of 200 mg/kg (III) and a diabetic group treated with a daily dosage of 400 mg/kg (IV). Each point represents mean ± SE for 8 rats.

Table 2 shows changes in fasting HbA1c levels after 28 days. The ethanol extract of *J. regia* had a significant effect on lowering HbA1c levels. Both the 200 mg/kg and 400 mg/kg treatments caused a decrease in glycolated hemoglobin and the extract with 400 mg/kg of *J. regia* brought glycolated

Table 2. The effects of *Juglans regia* leaves extract on glycosylated hemoglobin,triglyceride, cholesterol, LDL, VLDL, and HDL levels.

Group Parameters	Control I	Diabetic II	STZ+200 III	STZ+400 IV	F value df(3,20)
Glycosylated Hemoglobin (%Hb)	6.52 ± 0.12^{a}	12.33 ± 0.29^{c}	9.20 ± 1.11^{b}	6.41 ± 1.05^{a}	Sig 12.43
Triglycerides mg/dL	64.1 ± 4.72^{a,b}	85.2 ± 3.58^{c}	69.18 ± 3.52^{b}	57.1 ± 2.4^{a}	Sig 11.78
Cholesterol mg/dL	74.5 ± 3.66^{b}	84.1 ± 4.61^{c}	69.7 ± 3.77^{b}	56.9 ± 5.19^{a}	Sig 15.88
LDL mg/dL	33.22 ± 3.1^{b}	41.5 ± 2.6^{c}	22.95 ± 3.6^{a,b}	15.2 19.1 ± 33^{a}	Sig 7.82
VLDL mg/dL	11.74 ± 0.72^{a}	16.32 ± 1.45^{c}	13.54 ± 1.96^{b}	11.42 ± 0.98^{a}	Sig 13.39
HDL mg/dL	31.14 ± 1.49^{a,b}	27.2 ± 0.55^{a}	29.76 ± 1.33^{a,b}	31.52 ± 2.71^{b}	N S 4.87

The values are mean ± SE for 8 rats in each group. Means with different superscript (a, b, c, d) within a column are significantly different from each other at $P < 0.05$ as determined by Duncan's multiple range test.

hemoglobin levels close to those of the control group ($P < 0.05$).

In addition, Table 2 shows changes in fasting blood triglyceride levels after 28 days. Triglycerides were elevated significantly in the diabetic group (Group II) and brought to the same level as the control group in Group IV ($P < 0.05$). The ethanol extract of *J. regia* had a significant effect on lowering blood triglycerides.

Finally, Table 2 also shows changes in fasting total cholesterol, LDL, VLDL, and HDL levels after 28 days. In the diabetic group (Group II), total cholesterol, LDL, and VLDL levels were elevated after 28 days while the treatment brought Group IV's levels into line with the control group's levels. HDL levels did not change significantly in any of the groups studied ($P < 0.05$).

Figure 2 provides information about insulin levels in the experimental animals. Group I, the control group, did not show any significant variations in insulin levels. There was a significant decrease in

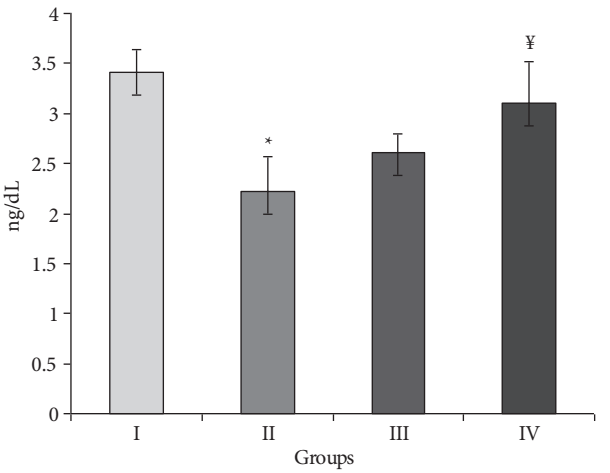

Figure 2. The effect of *J. regia* leaves extract on insulin levels (ng/dL) are shown for a control group (I), a diabetic group (II), a diabetic group treated with a daily dosage of 200 mg/kg (III) and a diabetic group treated with a daily dosage of 400 mg/kg (IV). The vertical bars are showing mean values of insulin levels (ng/dL). Lines above the bars indicate standard error (SE). The asterisk symbol (*) indicates that $P < 0.05$ when comparing Group II to Group I . The yen symbol (¥) indicates that $P < 0.05$ when comparing Group IV to Group II.

insulin levels in the diabetic group (Group II). However, the level of insulin was enhanced in Groups III and IV after treatment with the *J. regia* leaf extract. The insulin levels of Group IV animals almost reached that of those in the control group.

Discussion

The present observation of the hypoglycemic effect of *J. regia* leaves supports a number of other observations regarding the efficacy of plant extracts. Lemus et al. (20) reported on the hypoglycemic activity of dried leaves of the *Bauhinia ulrifolius*, *Galega officinalis, Morus alba,* and *Rubus ulnifolius* plants. They conducted short term experiments. Sachdewa and Khemani (21) reported on the hypoglycemic activity of an ethanol extract of the flower *Hibiscus rosa sinensis* on diabetes-induced rats. Andallu and Varadacharyulu (22) in their studies reported that fasting blood glucose levels were reduced in diabetic groups treated with mulberry. Mohammadi and Naik (23) reported that administering an extract from the *Morus alba* leaf for five weeks significantly improved hypertriglyceridemia and hypercholesterolemia. Komeili and Bagheri (24) in their studies reported that fasting blood glucose levels in diabetic groups treated with a *J. regia* extract were only reduced by 17% whereas in the present investigation both 200 and 400 mg/kg treatments restored the blood glucose level to that of the control group (Figure 1).

Throughout the circulatory life of red blood cells, glycohemoglobin is formed continuously by the addition of glucose to the N-terminal of a hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. Several investigators have suggested that glycosylated hemoglobin serves as an indicator of metabolic control of diabetes since glycohemoglobin levels approach normal values for diabetics in metabolic control (23,25,26). In the present investigation, glycosylated hemoglobin was elevated nearly 200% in the diabetic group (Group II). In Group IV, levels of glycosylated hemoglobin decreased to values that were close to those of the control group (Group I).

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. Increased levels of triglycerides are a risk factor for atherosclerotic coronary disease. Repeated administration of the *J. regia* leaf extract for 28 days significantly improved hypertriglyceridemia and hypercholesterolemia, bringing their levels in Group IV to those of the control group (Table 2). LDL and VLDL carry cholesterol to peripheral tissues where it can be deposited. Therefore, high levels of LDL and VLDL are atherogenic whereas HDL transports cholesterol from peripheral tissue to the liver,where it can be processed for excretion. Hence, HDL has a protective effect. In the present investigation, HDL levels did not change significantly in any of the groups of rats studied (Table 2). Tavakkoli et al. (27) have shown that replacing some fatty acids in the diet with walnuts for four weeks caused a decrease in total cholesterol and LDL cholesterol values among menopausal women , whereas in the present investigation all these parameters were reduced significantly when compared to the diabetic rats and ultimately reached the level of the control group.

The improvement in glycemic control followed by a decrease in VLDL production after *J. regia* treatment could be attributed to *J. regia* therapy in diabetic rats. Laakso et al. (28) and Laakso (29) showed improved glycemic control followed by a decrease in VLDL production in diabetic patients after being treated with oral agents. The overall effect of *J. regia* on VLDL metabolism could be due to a 2-fold course of action: reduction in VLDL production and enhancement of VLDL removal. Earlier studies of Laakso (29) and Taskinen (30) encountered a higher concentration of LDL cholesterol and lower concentration of HDL cholesterol in diabetic patients. Hypocholesteromic drugs decrease LDL cholesterol, presumably by stimulating receptor-mediated removal of LDL. This seems a likely explanation for *J. regia* treatment, which shows a decrease in LDL and no alteration in HDL-cholesterol control. The single dose STZ-induced diabetic rat is one of the animal models of human insulin dependent diabetes mellitus (IDDM) or type I diabetes mellitus. In this model, diabetes arises from irreversible destruction of the β-cells of the pancreas, causing degranulation or reduction of insulin secretion. In this type I model of diabetes, insulin is markedly depleted, but not absent (31). Although insulin has become one of the most important therapeutic agents known to medicine,

there is a continuing effort to find insulin substitutes, secretagogues, or sensitizers from synthetic or plant sources for the treatment of diabetes. Hypoglycemic effects have been reported for some plants that contain flavonoids (31), as *J. regia* does. The present study demonstrated that the alcoholic extract of *J. regia* has marked hypoglycemic property when given for 28 days to STZ-diabetic rats. Such an effect might be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, as has been noted, for example, when plant fiber is given orally with glucose (32). Hence, it may be presumed that the glucose-lowering effect of the plant extract was achieved by an extraintestinal action. Plasma insulin levels in Group IV were significantly higher on Day 28 compared to the untreated diabetic group (Group II). The elevation in plasma insulin levels in STZ diabetic rats treated with the alcoholic *J. regia* extract could be due to substances present in the plant extract which stimulate insulin secretion or which protect the intact functional β-cells from further deterioration so that they remain active and continue to produce insulin. Protection of the β-cells could also be, at least in part, a result of the reduction in blood glucose, thereby eliminating gluco-toxicity to the β-cells. This possibility is strengthened by the observation of insulin concentration.

In conclusion, our present investigation shows the therapeutic efficacy of *J. regia* leaves on diabetes-induced animal model at a treatment of 400 mg/kg per day.

Acknowledgements

The authors gratefully acknowledge the Yasouj University of Medical Sciences, Iran, for supporting this research and extending facilities.

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