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Hypoglycemic Activity of Fruits of *Juglans regia* L. on Streptozotocin Diabetic Rats*

Juglans regia L.Meyvalarının Streptozotocin Diabetik Sıçanlar Üzerindeki Hipoglisemik Etkisi

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Abstract

The leaves as well as the fruits of *Juglans regia* are widely used as traditional medicine in Turkey. In this study the hypoglycemic effect of this plant, used in the therapy of several diseases, was investigated. The extracts prepared from fresh fruits, were assayed on streptozotocin induced diabetic rats. The result obtained from the blood glucose levels, body weights, food intake and, histopatological examination of pancreatic tissue taken on 31st day under anesthesia from the rats, were compared with control values of normal rats and rats given glipizide. The methanol extract of *Juglans regia* fruits was found to have anti-diabetic activity.

Key words: *Juglans regia*, hypoglycemia, streptozotocin

Introduction

Juglans regia is a tree widespread throughout the world and reputed to possess several medicinal properties.

In Turkish folk medicine, the fruits and leaves of *J.regia* L. (walnut tree) have been widely used as an herbal remedy for the treatment of diabetes (Baytop, T.,1984; 1999; Öztürk Y. et al. 1994). Phytochemical studies of *J.regia* have revealed the presence of various compounds such as juglone, bisjuglone, trijuglone, naphthaquinone, naphthaquinol glucoside, naphthalenes, regiolone (Muller and Leistner, 1978; Talapatra *et al.*, 1988).

In order to understand the pharmacological basis the use in folk medicine of *J.regia* for the treatment of diabetes. This study was designed to investigate the effect of methanolic extract obtained from this plant on streptozotocin diabetic rats.

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Material and Methods

Plant materials

Unripe fruits of *Juglans.regia* L. (Juglandaceae) were collected from the countryside of Istanbul-Turkey in June 2000-2001.

Extraction

Juglans regia (10 fruits) were broken into pieces and kept at room temperature. They were immediately extracted in a Soxhlet apparatus with methanol (Merck) for 7 days. Combined methanolic extracts was evaporated under vacuum.

Dried extract (JRE) was dissolved in physiological saline solution prior to application to rats (100 mg/kg , i.p.)

Animals

Adult male Wistar albino rats (130-140g) were used in this study. They were housed in well-ventilated rooms. All rats were fed with standard diet and water ad libitium.

The rats were classified into three groups of 10 animals each.

I: Diabetic rats (with STZ), treated with saline (0.9% NaCl), i.p. for 30days

II: Diabetic rats, (with STZ), treated with plant extract, JRE (100 mg/kg), i.p. for 30days

III: Diabetic rats (with STZ), treated with standard hypoglycemic agent (Glipizide, Carlo-Erba), (10 mg/kg), i.p. for 30days

IV: Normal rats (with 0.9% NaCl), treated with saline (0.9% NaCl), i.p. for 30days

Streptozotocin-induced diabetes:

Streptozotocin (STZ) purchased from Sigma was dissolved in physiological saline solution immediately before use. The rats were anesthetized by ether and administred STZ (60 mg/kg).

Measurement of blood glucose, body weight, food and fluid intakes:

Body weight, food and fluid intakes were monitored daily during the experimental period. Blood samples for blood glucose determination were obtained from the tail tip of fasted rats. On weeks 0., 1., 2., 3., and 4. of the experiments blood glucose level was determined using glucostix-glucometer methods (Accutrend®alpha-Boehringer Mannheim).

Statistical analysis:

All the results were analyzed statistically using Student's t-test expressed as the mean±SD.

Histopathological examination:

The animals were sacrificed by ether anesthesia on the 31st day of experiment. All the tissue samples were formalin fixed and paraffin embedded for microscopic examination in accordance with routine laboratory procedures. Histological examination and grading were done on hematoxylin-eosin stained sections.The number of islets and the number of islet cells of each islet were counted.

Results

The blood glucose levels, food and fluid intake values increased significantly in streptozotocin treated rats **(Group I, II, and III)** compared the normal rats **(Group IV)**. Effects of the administration of JRE (100 mg/kg) to diabetic rats; change in body weights

and blood glucose levels were given **(Tab.1, 2; Fig.1-2)** ; food and fluid intake values were given **(Tab.3,4; Fig.3-4)**. Blood glucose levels in the rats given the JRE (100 mg/kg) were significantly reduced in the 3 th and 4 th weeks. During the 30 days of measurements, fluid and food intake values of STZ induced rats were determined and compared with the normal rats **(Group IV)**.

The results showed that, i.p. administration of STZ (60 mg/kg) effectively induced diabetes in the normal rats. This was defined by the body weight loss, high blood glucose values, more food and fluid intake. These values were compared with the values of the normal rats.

The *Juglans regia* fruit extract (JRE, 100 mg/kg) significantly inhibited the hyperglycemia of STZ induced rats. It was found that 100 mg/kg of JRE activity is very close to the activities of 10 mg/kg Glipizide (Table 2) (standard hypoglycemic agent). The results of histopathological examination are given in Table 5.

Table 1. Effect of *Juglans regia* extract (JRE) on body weight in rats

	Body weight (g)				
Group treatment	Initial	1.week	2.week	3.week	4.week
I STZ	138.88±18,33	137.5±34.01*	156.25±50.12*	155.25±53.8*	165±52.44*
II STZ+JRE	138.55±18.16	151.11±27.58*	178.57±29.68	181.42±30.78*	190±33.66
III STZ+ glipizide	137.77±22.79	155±22.03*	175±25.63*	175±27.77*	180±35.85*
IV Normal	131.42±24.10	192±19.23	208±19.23	216±19.49	230±14.14

Comparisons were made between:
(*)group IV and group I,II,III;
***The symbol represent statistical significance** (*): $p < 0.05$*

Table 2. Effect of Juglans regia extract (JRE) on blood glucose in rats

	Blood glucose (mg/dl)				
Group	Initial	1.week	2.week	3.week	4.week
I STZ	125.44±10.07	396.75±100.75*	399.62±69.27*	405.87±81.97*	412.42±111.65*
II STZ+JRE	117.77±6.99	395.66±114.71*	333.88±83.76*	285.71±82.54*$^{\#}$	285.28±74.40*$^{\#}$
III STZ+ glipizide	120.55±8.81	404±107.76*	339.25±101.92*	287.50±81.48*$^{\#}$	273.37±55.75*$^{\#}$
IV Normal	125.4±9.73	124.4±4.33	132.2±15.02	130.8±11.49	131.4±11.45

Comparisons were made between:
(*)group IV and group I,II,III; (#)group I and groupII,III
The symbol represent statistical significance
(*),(#) : $p < 0.05$

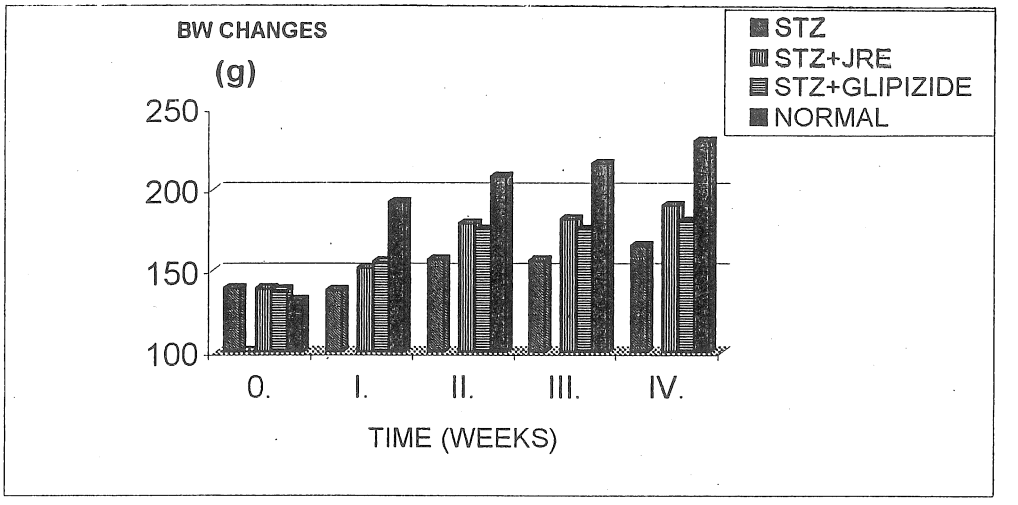

Figure 1. Effect of *Juglans regia* extract (JRE) on body weight in rats

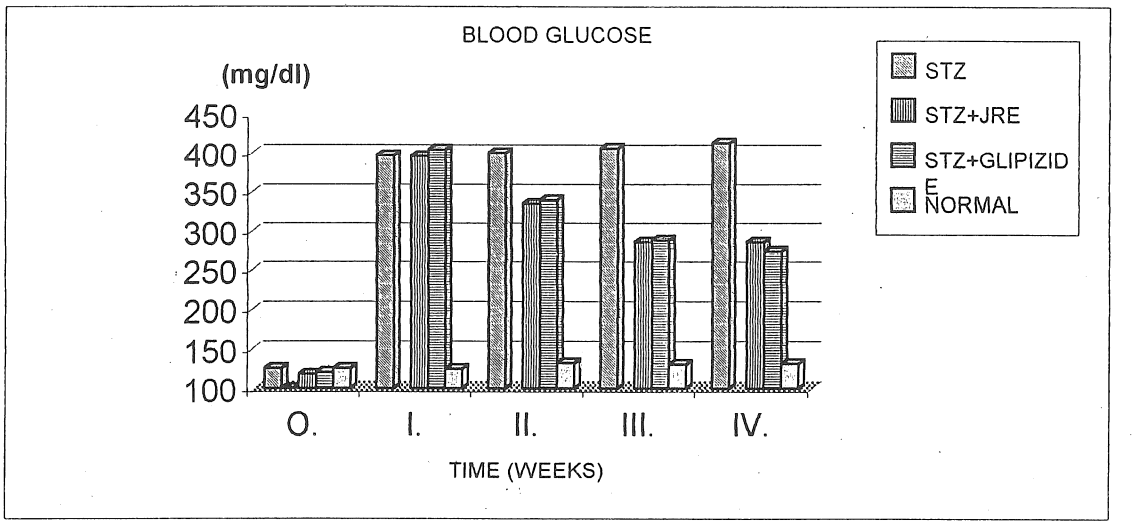

Figure 2. Effect of *Juglans regia* extract (JRE) on blood glucose in rats

Table 3. Effect of Juglans regia extract (JRE) on food intake in rats

Food intake (g/rat/ per day)	
Group treatment	
I STZ	18.65±2.09
II STZ+JRE	14.42±2.51
III STZ+ glipizide	15.38±1.49
IV Normal	13.11±2.25

Comparisons were made between:
No Significance

Table 4. Effect of Juglans regia extract (JRE) on fluid intake in rats

Fluid intake (ml/rat/ per day)	
Group treatment	
I STZ	35.26±5.27
II STZ+JRE	27.4±6.27
III STZ+ glipizide	29.46±2.33
IV Normal	17.23±4.04

Comparisons were made between:
No Significance

Table 5. Effect of Juglans regia extract (JRE) on the number of islets and the number of islet cells in pancreatic tissues in rats

Group treatment	Islets	cells
I STZ	15.83±7.22*	59.45±15*
II STZ+JRE	16.57±8.89*	97.47±34.92#
III STZ++ glipizide	18.5±9.77	82.65±20.3#
IV Normal	27.6±6.66	89.52±24.8

Comparisons were made between:
(*)group IV and group I,II,III; (#)group I and groupII,III
The symbol represent statistical significance (*),(#) : p <0.05

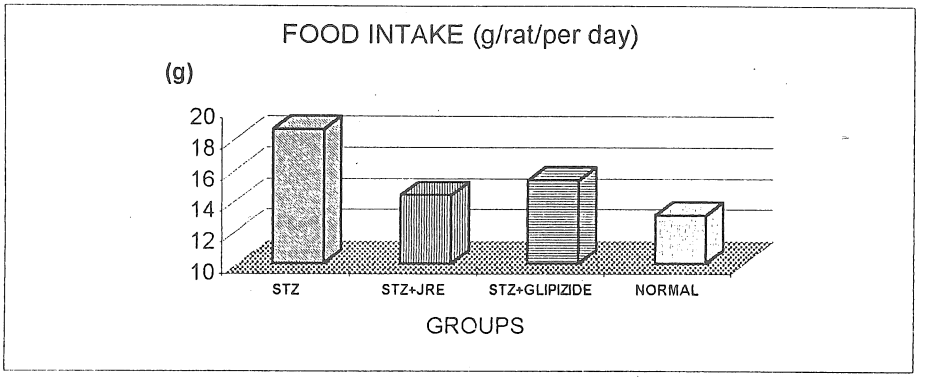

Figure 3. Effect of *Juglans regia* extract (JRE) on food intake in rats

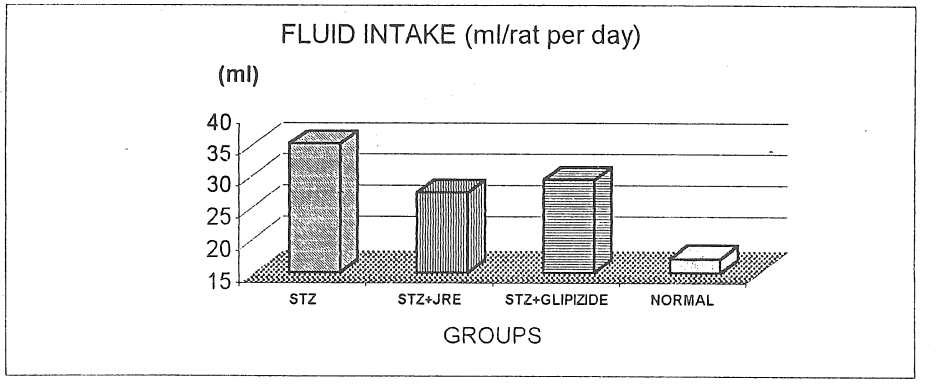

Figure 4. Effect of *Juglans regia* extract (JRE) on fluid intake in rats

Discussion

The results show that i.p. administration of STZ (60 mg/kg) effectively induced diabetes in normal rats. The methanolic extract of *J.regia* fruit extract (100 mg/kg) significantly

inhibited the hyperglycemic action of STZ. (Table 1) and significantly enhanced the number of islets and cells of the pancreas in diabetic rats (Table 5).

Our results suggest that the *J. regia* fruit extract can be important agent against STZ induced diabetes in rats. This effect can be attributed to compound such as juglon and its derivatives present in this plant extract. These compounds can be potentiating the insulin effect of plasma by increasing the pancreatic secretion of insulin from the β cells of islets or release the insulin from the bound insulin.

Further studies on the isolation of active constituent(s) responsible for the anti-diabetic activity and clinical investigations, are currently under progress in our laboratory.

Özet

Ceviz ağacının gerek yaprakları gerekse meyvaları ülkemizde çok kullanılan bir halk ilacıdır. Çeşitli hastalıkların tedavisinde kullanılan bu bitkinin özellikle kan şekerini düşürücü etkisi bu çalışmada incelenip, irdelendi.

Taze meyvalardan hazırlanan ekstreler, streptozotocin maddesi ile diabet yapılan sıçanlar üzerinde denendi. Sıçanların kan şekeri düzeyleri, vücut ağırlıkları ile sıvı ve yem alma değerleri ve 31.günde anestezi altında sıçanlardan alınan pankreas dokusunda yapılan histopatolojik incelemelerden elde edilen veriler, normal sıçanlar ve kontrol maddesi olarak glipizid verilen sıçanlar ile karşılaştırılarak değerlendirildi. Sonuç olarak ceviz meyvalarının kan şekerini düşürücü etkisi doğrulandı.

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