

## Effects of a Hydroalcoholic Extract of Walnut Male Flowers on Diabetic Rats

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Article information	Abstract
<p>Article history: Received: 10 July 2012 Accepted: 20 Nov 2012 Available online: 15 Jan 2013 ZJRMS 2013; 15(11): 55-58</p> <p>Keywords: Diabetes Walnut male flowers Rats Hydroalcoholic extract Liver function test</p> <p>*Corresponding author at: Department of Biology, Science and Research Branch, Islamic Azad University, Fars, Iran. E-mail: ebrahim.hosseini@yahoo.com</p>	<p><b>Background:</b> Diabetes is a metabolic disorder resulting from defects in insulin secretion and function. Walnut is used in traditional medicine to treat diabetes. In this study we evaluated the anti-diabetic effects of the hydroalcoholic extract of walnut male flowers in streptozocin diabetic rats and its probable side effects on the liver.</p> <p><b>Materials and Methods:</b> Eighty adult male Wistar rats were randomly selected and divided into 4 subgroups including a control (N=8) with no intervention, witness group receiving normal saline and another 3 groups of rats each receiving either 2, 4, or 6 g/kg of the extract per day for 15 days. Diabetic groups of rats each treated with the above doses of the extract for the aforementioned period of time, and a group of 8 diabetic rats without any further treatment. Eight rats were also used to determine the LD50 of streptozotocin. Diabetes was induced in rats by injection of 60 mg/kg of streptozotocin. At the end of the experimental period, blood was taken from the experimental and control groups and the serum levels of insulin, glucose and liver enzymes (ALT, AST, ALP) were measured.</p> <p><b>Results:</b> Results showed that the hydro-alcoholic extract of walnut male flowers increased the levels of insulin, decreased blood glucose, AST and ALP enzymes in the treated diabetic groups compared to the non-treated group (<math>p&lt;0.05</math>). The anti-diabetic effects of the extract were not dose dependent.</p> <p><b>Conclusion:</b> The effectiveness of the hydro-alcoholic extract of walnut male flowers in diabetic rats through prevention of liver damage and reduction of blood glucose.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

### Introduction

Diabetes mellitus is a common metabolic disorder with hyperglycemic symptoms [1]. Diabetes symptoms are either due to a decreased secretion of insulin or an increased resistance of target cells to this hormone or both. There are many different complications of this autoimmune disease including changes in the intracellular metabolism of tissues such as increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the liver and blood [2, 3]. Blood glucose concentration is the most important factor in regulating insulin secretion from  $\beta$ -cells of pancreas. Glucose is carried by blood to pancreas by GLUT-2 to regulate insulin secretion from pancreas [4]. Production of ATP from glucose will also regulate ATP sensitive potassium channels which regulate insulin secretion. This leads to membrane depolarization and opening of voltage-dependent calcium channels in the  $\beta$  cell membranes and the increase of calcium entry into the cells, resulting in insulin secretion [5].

Liver is one of the main targets of insulin and plays an important role in maintaining stable blood glucose levels and is also the main site of detoxification of drugs and metabolites. Liver can inactivate drugs and metabolites by enzymatic reactions, especially through transaminases [6]. During chronic liver disease, muscular dystrophy and chronic renal disease, serum levels of liver enzymes such as AST (aspartate aminotransferase) and ALT (alanine

aminotransferase) will increase in the liver and plasma [7, 8].

It has been shown that the number of patients with diabetes will increase in future from 150 million in the year 2000 to 300 million in the year 2030 [9, 10]. Therefore, using herbal medicines with no side effects on liver are of interest for the treatment of diabetic patients [11]. Different parts of walnut tree are currently used in traditional medicine. In the current study the effect of a hydroalcoholic extract of walnut male flowers on the level of blood glucose and insulin in streptozotocin diabetic rats is studied. Liver protecting properties of this extract were also checked by measuring the serum levels of ALT, AST and ALP enzymes in diabetic rats treated with this extract.

### Materials and Methods

This study is an empirical study was performed at Islamic Azad University of Fars Science and Research Branch and Shiraz University of Medical Sciences in 2011.

**Preparation of walnut male flower extract:** Walnut male flowers were collected from walnut trees, dried and pulverized. Twenty grams of the dried powder were mixed with 300 ml of 50% hydroalcoholic solution in a percolating container and incubated for 72 hours at room temperature. Then the extract was separated and heated

for 12 hours at 50°C and finally dried in a dessicator for 24 hours.

**Animal experiments:** Eighty adult male Wistar rats with an average weight of 200-225 grams were used in this study using optimal living conditions. During the period of drug administration all of the animals used the same food and water without any restrictions in a condition of 27±2°C temperature with 12 hours of natural light and 12 hours of darkness. The study protocol was based on the international laws on laboratory animals and was approved by the University Ethics Committee. Rats were subdivided into 10 groups of 8 rats for our experiments. One group was used as a control, 4 groups were made diabetic with streptozotocin (see below), 4 groups were kept as non-diabetic for studying the effects of the various doses of the extract on normal rats and a group was used for the determination of the LD50 of the extract. Diabetes was induced in animals by an intraperitoneal injection of 60 mg/kg of streptozotocin to each rat (Kalamazoo, MI, USA) [12]. After 3th and 7th days, blood samples were taken from the animals and glucose was measured. Rats with blood glucose concentrations of more than 300 mg/dl were considered as diabetic. Each experimental group was divided into 4 subgroups: the control group received normal saline and the other three groups received 2, 4 or 6 g/kg of the extract in normal saline. To determine the lethal dose of the extract, 8 rats were administered with up to 40 g/kg dose of the extract. After the final day of the experiments (16th days), the rats were anesthetized and blood sampling was performed. All separated sera were stored

at -20°C until use. Glucose, ALT, AST, ALP (Pars Azemooon, Tehran, Iran) and insulin (DRG International Inc., USA) levels were measured according to the manufacturer's instructions.

The data were analyzed by one way ANOVA, Tukey post-hoc test and Mann-Whitney *U* using SPSS-18 statistical package (SPSS Inc, Chicago, IL, USA). *p*-value less than 0.05 was considered significant.

## Results

Our results showed that the administration of hydro-alcoholic extract of walnut male flowers to mature male diabetic rats results in a significant increase in blood insulin (Table 1) and a reduction in blood glucose level (Table 2) compared to the sham control groups. No changes were observed in the levels of insulin and blood glucose in non diabetic rats (Tables 1, 2).

Our results showed that the induction of diabetes in rats resulted in significant increases in serum levels of AST and ALP enzymes compared with the control groups. Oral administration of the hydroalcoholic extract of walnut male flowers for 15 days reduced the levels of these enzymes in diabetic rats compared to the control group (*p*<0.05), but this extract had no effects on the level of serum ALT. Similarly, no significant differences were observed in the serum levels of AST and ALP enzymes in non-diabetic group treated with different doses of the extract (Table 3).

**Table 1.** Comparison of the serum insulin levels in diabetic and non-diabetic groups treated with the hydro alcoholic extract of walnut male flowers

Groups	Serum levels of insulin (Mean±SD) (µg/l)				
	Control	Sham	Experimental groups (treated with the flower walnut flower extract)		
			2 g/kg	4 g/kg	6 g/kg
Diabetic	0.62±0.15	0.03±0.02	1.31±0.63*	1.15±0.71**	1.68±1.01***
Non-diabetic	0.62±0.15	1.16±0.41	1.40±0.74	1.46±0.81	1.81±0.98

\**p*≤0.05, \*\**p*≤0.01, \*\*\**p*≤0.0001

**Table 2.** Comparison of serum glucose levels in diabetic and non-diabetic groups treated with a hydro alcoholic extract of walnut male flowers

Groups	Serum glucose level (Mean±SD) (mg/dl)				
		Diabetic		Non-diabetic	
		First day	Fifteenth day	First day	Fifteenth day
Control		142.5±16.7	147.5±17.5	142.5±16.7	147.5±17.5
Sham		528.1±101.0	673.1±92.2	151.3±15.1	158.8±15.8
Experimental groups	2 g/kg	744.4±100.5	362.5±204.8*	156.9±8.4	154.4±14.5
	4 g/kg	685.0±139.2	430.0±199.7*	156.8±8.3	160.6±9.8
	6 g/kg	595.0±103.9	356.0±151.4*	155.6±7.8	158.9±9.2

\**p*≤0.0001

**Table 3.** Comparison of serum levels of ALP, AST, and ALT enzymes in diabetic and non-diabetic groups treated with the hydroalcoholic extract of walnut male flowers.

Groups	Serum levels of liver enzymes (Mean ± SD) (IU/L)						
		Diabetic			Non-diabetic		
		ALP	AST	ALT	ALP	AST	ALT
Control		13.6±3.2	136.3±24.6	63.5±2.7	13.6±3.2	136.3±24.6	63.5±2.7
Sham		14.1±2.9	177.5±14.1**	75.6±12.9	10.6±1.7	135.6±18.6	59.4±6.8
Experimental groups	2 g/kg	9.0±2.7**	135.6±29.2**	64.4±21.9	9.0±1.4	126.9±26.0	59.4±18.0
	4 g/kg	9.0±2.7**	137.5±30.5*	72.5±17.7	9.3±1.8	118.1±26.2	59.4±18.1
	6 g/kg	10.5±2.5*	126.3±10.9**	73.1±20.7	8.6±1.2	121.3±24.6	62.8±16.9

\**p*≤0.05, \*\**p*≤0.005

## Discussion

Results showed that the hydro-alcoholic extract of walnut male flowers increased the levels of insulin, decreased blood glucose, liver AST and ALP enzymes in the treated diabetic groups compared to the non-treated group ( $p < 0.05$ ). The anti diabetic effects of the extract were not dose dependent.

Type 2 diabetes or non-insulin dependent diabetes is characterized by chronic hyperglycemia, resulting in increased production of reaction oxygen species (ROS). In addition, increasing the glycosylation of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase will reduce their activity required for the destruction of ROS and will result in secondary disorders of diabetes and eventually will cause tissue damage [13]. The liver is one of the main targets of insulin, and liver damage is one of the consequences of diabetes resulting in increased levels of ALT, AST and ALP enzymes in blood [14]. This is probably due to increased permeability of cell membrane in the early stages of liver damage as well as increased protein catabolism associated with gluconeogenesis and urea formation [15].

Currently, insulin injection is the main treatment for blood glucose reduction in diabetic patients. Certain other commercial products are also used for the treatment of diabetes [16]. These compounds have undesirable side effects such as increasing the fat stores, loss of adipose tissue at the injection site and production of hypoglycemic shock [16]. Due to the high costs of medication and their side effects, the need for new and effective treatments with fewer side effects are quite evident [17, 18].

Herbal medicines are a proper replacement, as they have less cost and lower side effects. Walnut (*Juglans regia*) other than being used as a nutritious nut in our every day food, several parts of this tree have been used as drugs in traditional medicine. The leaves of this plant are used for the treatment of rheumatic pains, fever, anemia and diabetes [19].

Other studies have shown that the roots of the walnut tree are effective for the treatment of diabetes, malaria, rheumatic pains and removal of kidney stones [20, 21]. Based on the important role of ROS in the streptozocin-induced diabetes mellitus [22], fortification of the antioxidant systems of cells in diabetics could have an important and effective role in preventing or reducing the side effects of diabetes and its complications [13].

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Many studies have shown that walnut leaves have phenolic compounds and antioxidants such as flavonoids and chlorogenic acid [23]. Studies also have shown that flavonoids can reduce blood sugar by inhibiting the intestinal absorption of glucose and also chlorogenic acid by the inhibition enzyme of glucose-6 phosphatase (Glc-6-P), as key factors in regulating blood sugar [24, 25]. It is suggested that the flavonoids and their antioxidant properties in the hydroalcoholic extract of walnut leaves can be effective in the treatment and prevention of diabetes [26].

In agreement with our findings, Fathiazad and Garjani have shown that hydroalcoholic extract of walnut leaves can reduce blood sugar in streptozocin-induced diabetic rats, without any effect on the control animals [27]. Also it has been demonstrated that the hydroalcoholic extract of walnut leaves can prevent liver damage following diabetes and it has a significant effect on the reduction of serum AST and ALT enzymes [28].

On the other hand, the ethanolic extract of walnut leaves decreases blood glucose, cholesterol, triglycerides, urea nitrogen, creatinine, AST, ALT and ALP enzymes in alloxan diabetic rats [29]. Extracts of walnut leaves are effective in the reduction of blood sugar and fat, and have healing effects on metabolic disorders caused by diabetes in rats [30, 31].

In conclusion, our results showed that the hydroalcoholic extract of walnut male flowers can increase serum levels of insulin, and decrease blood glucose levels in diabetic rats. More investigations are needed to explore the anti diabetic effects of walnut male flowers in human

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## Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

## Conflict of Interest

The authors declare no conflict of interest.

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