The effect of hydro alcoholic extract of *Juglans regia* leaves in streptozotocin-nicotinamide induced diabetic rats

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Abstract: Phytotherapy has been achieved to maintain glycemic control in patients with diabetes mellitus. The present study was conducted to evaluate the antihyperglycemic properties of the *Juglans regia* leaf extract in streptozotocin-nicotinamide induced diabetic rats. Nicotinamide was injected intraperitonealy (i.p.) 15 min before the injection of Streptozotocin (i.p.). One week after induction of diabetes, oral treatment started with extract of *Juglans regia* and Metformin and continued for 4 weeks. Fasting blood sugar, body weight, serum lipids and insulin level were measured in different groups. A significant reduction of glucose, HbA1c, total cholesterol and serum triglycerides were detected after 4 weeks in rats treated with *Juglans regia* leaves compared to the control groups. Thus, *Juglans regia* extract treatment showed potential hypoglycemic and hypolipidemic effects in type 2 diabetic rats.

Keywords: *Juglans regia*, glucose, serum lipids, Streptozotocin, Nicotinamide, type 2 diabetes.

INTRODUCTION

It is predicted that the type 2 diabetes is rising dramatically and reaches 370 million worldwide for the year 2030 (Smyth and Heron, 2006). In most cases diabetes mellitus is accompanied with retinopathic, neuropathic, nephropathic and atherosclerosis diseases and which is a common debilitating condition (Jarvisalo *et al.,* 2002). The rate of coronary heart disease (CHD) is more likely to develop in diabetic patients than non-diabetics (Bolen *et al.,* 2007; Mohammadi and Naik, 2008). So, in past decades, different treatments were used to cure of diabetes mellitus were used, including synthetic drugs and phytotherapy.

Although, these antidiabetic synthetic drugs have been used for diabetes mellitus treatment in past decades, but most therapeutic goals have not been achieved for most patients. Therefore, a novel approach for cure of type 2 diabetes is necessary. Studies have shown that several plant extracts reduced the lipid profiles and coronary heart disease in diabetic rats (Khan *et al.,* 2003: Chakrabarti *et al.,* 2003). There is evidence that the *Juglans regia* (*J. regia*) could be an alternative treatment for diabetes mellitus (Bolen *et al.,* 2007). *J. regia* is a high sized tree and distributed almost over Zagros area in Iran. It is a deciduous tree of the *Juglandaceae* family and its leaves have been widely used in traditional medicine. *J. regia* has been used to treat skin inflammations, hyperhidrosis, ulcers, diarrheic, helmintic, septic and astringent properties (Bruneton, 1999; Proenca da Cunha *et al.,* 2003). Metformin is the common drug and one of the mainstays of therapy for type 2 diabetes. Also, it can be used either as initial therapy or as a combined drug with other antidiabetic agents (Fonseca *et al.,* 2000). So far there is not enough evidence regarding to the efficiency of the antidiabetic property of *J. regia.* Thus, this investigation was conducted to determine the effect of *J. regia* in streptozotocin-nicotinamide induced diabetic rats.

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MATERIALS AND METHODS

Sample preparation and extraction procedure

The *J. regia* leaves were collected during November 2009 from gardens located in east part of Yasouj City, Iran. The authentication of the plant sample was confirmed by a taxonomist in the Botany Department and voucher specimen was 2-10 HMRC. The leaves of healthy plants were plucked, washed thoroughly under running tap water, dried outside in the shade for 5 days and then ground into the fine powder using an electric mixer. The powdered plant material (700 g) was soaked in 90% ethanol at room temperature for 24 hours, a procedure repeated twice. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was evaporated in the soxhlet apparatus to obtain 80.4 g extract powder. The extract was stored in a refrigerator at 2–8^{o}C to be used in subsequent experiments.

Induction of non-insulin-dependent diabetes mellitus (NIDDM)

NIDDM was induced in overnight fasted male adult Wistar rats (6 weeks age, 160-200g) following the method used earlier (Pellegrino *et al.,* 1998; Shirwaikar *et al.,* 2004). For this purpose, streptozotocin (Sigma Aldrich, Germany) and nicotinamide (Ranbaxy Chemicals Ltd.,

India) were dissolved in citrate buffer (pH 4.5) and normal saline, respectively. Nicotinamide was injected (120 mg/kg, i.p.) 15 min. before the injection of Streptozotocin (60 mg/kg, i.p.). Plasma glucose levels were measured at 72 h and 7th day after injection. Diagnosis of type 2 diabetes mellitus determined based on fasting plasma glucose concentration of FPG ≥126 mg/dL (American Diabetes Association, 1998).

Experimental design

Forty eight Wistar rats (6 weeks age, 160-200g) were procured from the animal house of the Yasouj University of Medical Sciences (YUMS). All experiments were performed according to the local ethical committee in YUMS, for use and care of animals by the code number 88.11.17.12. The animals were maintained under the standard conditions based on *ad libitum* at room temperature 20±5°C with a regular 12: 12 h L/D cycle. The animals were divided randomly into six groups of eight animals. Groups I and II received 1ml distilled water and *J. regia* alcoholic extract (200mg/kg), respectively. Group III; diabetic control rats received 1 ml distilled water; Group IV; diabetic rats treated with *J. regia* daily (200 mg/kg); Group V; diabetic rats treated with *J. regia* alcoholic extract (400 mg/kg); Group VI; diabetic rats treated with Metformin (500 mg/kg). One week after the induction of diabetes all rats treated daily for 28 days. Various biochemical parameters of serum and fasting plasma glucose levels (Nicholas, 1956) were determined at the end of the study. Serum glucose levels were measured during the study, using GOD- POD kit (Zistchimie, Iran) on zero day (before the experiment) and seventh day of every week (Trinder, 1969). Serum insulin level was measured using ELISA kit (Boeheringer, Germany). Serum lipid profiles and HbA1c levels were estimated by an autoanalyser (Hitachi 912, Germany). The body weight was recorded at the first and final day of experiment.

STATISTICAL ANALYSIS

One-way ANOVA was used for data analysis, followed by Duncan's multiple range test. The values were considered significant when $P<0.05$.

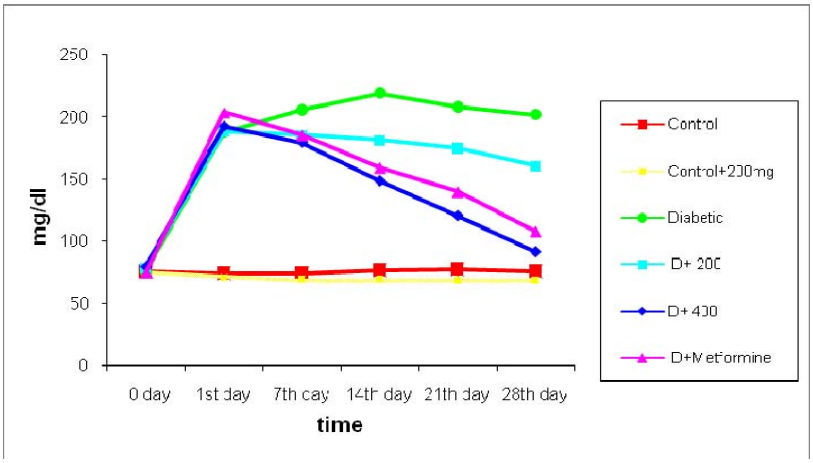

Fig. 1: The effect of alcoholic extract of *J. regia* on fasting plasma glucose level in different groups. Each points represents mean ± SE for 8 rats in group. Values compared within the all groups by Duncan's multiple range test. P-value<0.05 was considered to be statistically significant

Table 1: Effect of alcoholic extract of *J. regia* on serum lipid profiles and HbA1c levels in different groups

Groups / Parameters	Control I	Control + 200 mg/kg II	Diabetic control III	D +200mg/kg IV	D +400 mg/kg V	D + metformin VI
Triglyceride (mg/dl)	75.28 ± 1.77	74.21 ± 2.91	159.66± 8.45	135.32±3.61[a]	89.30± 4.70[a]	81.36± 4.37[a]
Total Cholesterol (mg/dl)	63.11 ± 3.41	61.36 ± 2.65	115.73± 9.88	71.33± 5.26[a]	61.28± 3.11[a]	64.27± 1.79[a]
LDL(mg/dl)	56.28 ± 4.62	55.34 ± 2.73	126.45±7.73	118.51± 5.22	76.29± 3.44[a]	74.57± 3.27[a]
VLDL(mg/dl)	15.32 ± 1.66	14.82 ± 2.64	33.52 ± 7.21	23.88± 6.35[a]	18.41± 3.28[a]	16.03± 2.81[a]
HDL(mg/dl)	24.45 ± 3.65	23.09 ± 1.73	17.55 ±1.36	19.45 ± 2.47	23.45± 3.29[a]	21.07± 1.51[a]
HbA1c (%)	6.46 ± 0.13	6.20 ± 0.07	13.58±0.15	11.61±0.13	8.29±0.12[a]	6.38±0.04[a]

Data are means±SE (n = 8 rats/group). a Significant different from treatment groups vs. diabetic control ($P < 0.05$).

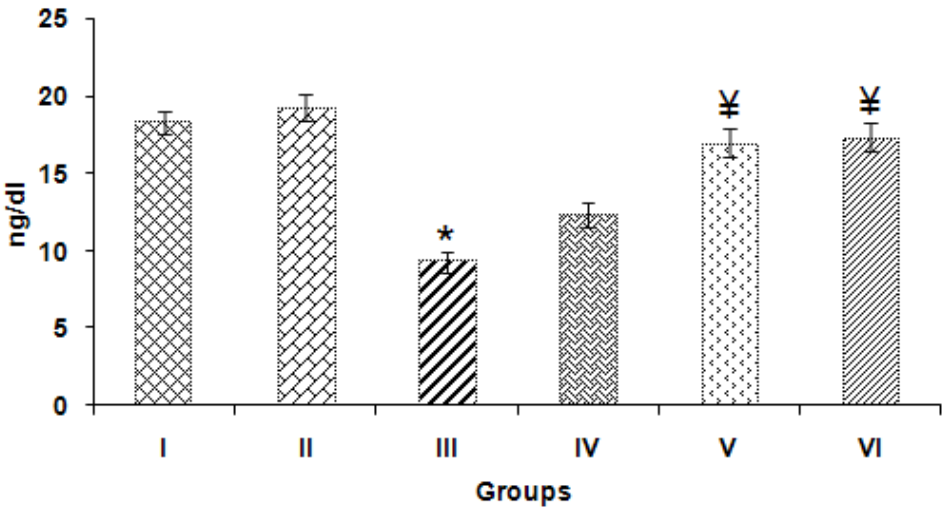

Fig. 2: The effect of alcoholic extract of *J. regia* on serum insulin levels in different groups. Values are given as mean ± SE for groups of 8 rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the all groups by Duncan's multiple range test. Lines above the bars indicate standard error (SE). The asterisk symbol (*) indicates that $P < 0.05$ when comparing Group III to Group I. The yen symbol (¥) indicates that $P < 0.05$ when comparing Group V and VI to Group III.

RESULTS

Results showed that the *J. regia* has significant impact on type 2 diabetes and there was no evidence of toxic reaction during this study. Fig. 1 shows the fasting plasma glucose levels in different groups. The mean of fasting plasma glucose decreased in animals treated with *J. regia* (V) and Metformin (VI) compared to the diabetic control group ($P<0.05$). The serum triglycerides, total cholesterol, HbA1c levels decreased and HDL cholesterol levels increased significantly in groups V and VI in comparison to diabetic control rats $P < 0.05$ (table 1). There was also a significant increase in insulin level in group V (400 mg/kg) and VI (Metformin) compared to the diabetic control rats $P<0.05$ (fig. 2). The body weight increased significantly in animals with *J. regia* therapy compare to the diabetic control group (table 2).

Table 2: The effect of alcoholic extract of *J. regia* on body weight levels in different groups

Parameter / Groups	Initial weight (g)	Final weight (g)
Control I	175.76 ± 1.72^{b}	201.46 ± 3.64^{d}
control + 200 mg/kg II	173.59 ± 4.76^{b}	205.28 ± 6.41^{d}
Diabetic control III	171.48 ± 8.41^{b}	145.66 ± 3.79^{a}
D +200 mg/kg IV	172.25 ± 3.97^{b}	167.43 ± 4.61^{b}
D +400 mg/kg V	176.45 ± 5.73^{b}	189.45 ± 2.75^{c}
D + metformin VI	169.65 ± 6.83^{b}	182.35 ± 5.54^{c}

DISCUSSION

The increase level of glucose and insulin in diabetic group demonstrated the induction of diabetes by streptozotocin-nicotinamide in rats. Following the induction of diabetes, the β cells showed degenerational changes in rats. Therefore, release of insulin is decreased in pancreas (Yin *et al.,* 2006; Rajalakshmi *et al.,* 2009). This study showed that, the alcoholic extract of *J. regia* has protective effect on streptozotocin-nicotinamide induced type 2 diabetic rats. Streptozotocin-nicotinamide injection can give rise diabetes mellitus, and ruin the β-cells in the islets of Langerhans (Kavalali *et al.,* 2002). The increase of blood glucose could damage β cells and lead to reduction in consumption of glucose by muscle tissues (Ghosh, 2004). This study demonstrated that the use of *J. regia* increased glucose utilization in muscle and resulted in a decrease of fasting plasma glucose in animals. The possible mechanism in why the *J. regia* brings hypoglycemic action in diabetic rats could be explained by its potential positive effect on repairing or regeneration of existing β cells (Bolen *et al.,* 2007; Moqbel *et al.,* 2011). The injection of *J. regia* with 400mg/Kg has the same effect as the Metformin on improving insulin secretion. Furthermore, the synthetic drug has many side effects, whereas, our results showed no any reaction in the body or uptake of food and water during the study. Therefore the decrease of blood glucose is due exclusively to the *J. regia* extracts in V group. Our result also indicated the deficiency of insulin hormone throughout the experiment in diabetic group without any treatment. In accordance with the earlier report (Teimori *et al.,* 2010), our results also showed that the *J. regia* leaves are effective substance in balancing glucose in diabetic rats. It has been

shown that the stem bark of *J. regia L* contains antioxidant agents (Kale *et al*., 2011) that could potentially inhibit secondary damage to β cells and be effective in the repairing process. In normal condition the insulin activates lipoprotein lipase and hydrolyses triglycerides, but insulin secretion deficiency results in an increase in hypertriglyceridemia. After induction of diabetes the inflammation of the islets of Langerhans have a key role in insulin secretion (Asgary *et al.,* 2008). It may be the antioxidant property in *J. regia* leaves have anti-inflammatory effect. Further, histological study has indicated that the *J.regia* could control the size of islets of Langerhans in diabetes –induced rats (Asgary *et al*., 2008). Also, it has been shown that improvement in insulin secretion in the islets of Langerhans leads to decrease of the serum lipids levels (Asgary *et al.,* 2008; Mohammadi *et al.,* 2011). In contrast, our experiment has demonstrated that the HbA1c levels increased significantly ($p<0.05$) in diabetic group compare to the control. Furthermore, *J. regia* can prevent elevation of HbA1c significantly, and thereby decreases its level in diabetic rats and it is more likely, the *J. regia* have efficacy on glycemic control. The HbA1c is considered to be the best indicator of glycaemia (Mohammadi and Naik, 2008; Koenig *et al.,* 1976). Moreover, it was found that the HbA1c elevated significantly in diabetic animals, and this is directly related to the fasting blood glucose level (Mohammadi and Naik, 2008; Koenig *et al.,* 1976). This could be due to the effect of improved glycemic control created by *J. regia*. The induction of diabetes with STZ leads to reduction of body weight, which may be correlated to the muscular atrophy (Swanston-Flat *et al.,* 1990) and due to loss of tissue proteins (Chatterjea and Shinde, 2002). Our findings showed, the body weight was gain in alcoholic extract and Metformin treated diabetic rats. Several researchers reported, streptozotocin diabetic rats treated by herbal extracts showed increase in body weight compare to the diabetic control rats (Grover *et al*., 2002, Prasad *et al*., 2009). This could be due to increased in muscle glucose uptake which results in preventing tissue loss.

CONCLUSION

It could be claimed that traditional use of *J. regia* as a hypoglycemic agent, is effective nearly the same as Metformin medicine. However, more studies such as isolating the ingredients of the compound are needed to understanding the exact mechanism of *J. regia* on diabetes mellitus disease.

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