Effects of *Juglans regia* L. leaf extract on hyperglycemia and lipid profiles in type two diabetic patients: A randomized double-blind, placebo-controlled clinical trial


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**A R T I C L E   I N F O**

**Article history:**
Received 12 June 2013
Received in revised form 27 December 2013
Accepted 14 January 2014
Available online 23 January 2014

**Keywords:**

*Juglans regia*

Diabetes

Medicinal plants

Blood glucose

Lipid profiles

**A B S T R A C T**

**Ethnopharmacological relevance:** The *Juglans regia* L leaf has been traditionally used for treatment of diabetes mellitus in Iran. But yet, no controlled human study has determined its efficacy in diabetic patients. The present study was designed to investigate the effects of the *Juglans regia* leaf extract on hyperglycemia and lipid profiles in type II diabetic patients.

**Materials and method:** Total 61 patients, suffering from type II diabetes with fasting blood glucose (FBG) between 150 and 200 mg/dL, glycated hemoglobin (HbA1c) between 7% and 9% and aged between 40 and 60 years were selected, and randomly divided into two groups of *Juglans regia* and placebo. First group received 100 mg *Juglans regia* leaf extract in capsules form two times a day for 3 months and other group received 100 mg placebo capsule with the same dosage. The standard anti-diabetic therapy (metformin and glibenclamide, and nutritional regimen) was continued in both groups. At the baseline and after three months the FBG, insulin, HbA1c, cholesterol, triglyceride, HDL, LDL and liver and renal function tests were determined. In addition general satisfaction with the treatment was identified using health questionnaires.

**Results:** The results indicated that FBG, HbA1c, total cholesterol and triglyceride levels in *Juglans regia* treated patients significantly decreased compared with the baseline and with placebo group. Patients in *Juglans regia* group were significantly satisfied with *Juglans regia* treatment compared with the placebo group. No liver, kidney and other side effects were observed in the groups, except more GI events (specifically a mild diarrhea) associated with extract treatment at the beginning of the study.

**Conclusion:** In conclusion, treatment of type II diabetic patients with 100 mg *Juglans regia* leaf extract two times a day for three months improves lipid profile and glycemic control without any tangible adverse effects.

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1. Introduction

Type II diabetes which is characterized by high blood glucose in the context of impairment of insulin secretion and defects in insulin action is becoming a major worldwide health problem (Norris et al., 2001). According to a survey performed in 2012, the number of people living with, and dying of, diabetes across the world is quite shocking. Approximately, 346 million people worldwide have diabetes, and among which 90% of all cases suffer from Type II diabetes (Scully, 2012). Most mortality among diabetic patients is the result of cardiovascular disease and this may be largely the result of increased atherosclerosis following by hyperlipidemia. As a consequence of insulin resistance, type II diabetes is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced HDL, a predominance of LDL, and elevated triglycerides (Pradhan et al., 2001; Krauss, 2004). Furthermore, it is well established that antioxidant drugs can attenuate hyperlipidemia related to type II diabetes (Evans...
et al., 2002). In view of the increasing prevalence of diabetes and the burgeoning cost of managing patients, effective anti-diabetic drugs are still in great demand. The limited efficacy and unwelcome side effects of anti-diabetic drugs draw both patients and scientist's attention to alternative therapy including herbal medicine. *Juglans regia* L. leaves have long been used in Iran in the treatment of several diseases including diabetes (Haji Sharifi, 2003; Mirheidar, 2007). *Juglans regia*, is Persian or English walnut tree species, belongs to the family Juglandaceae and native to the region stretching from the Balkans eastward to the Himalayas and southwest China. It is a large, deciduous tree attaining heights of 25–35 m, and a trunk up to 2 m diameter (Zargari, 2012). Historically, several parts of plant including seeds, bark, leaf, and seeds green husks are used as natural remedies in folk medicine (Haji Sharifi, 2003; Zargari, 2012). *Juglans regia* seed is important as a dietary source of protein, fiber, essential fatty acids, vitamins, minerals and a good source of a wide variety of flavonoids, phenolic acids and related polyphenols (Caglarirmak, 2003). Leaves and green husk in *Juglans regia* are a source of phenolic compound ferulic acid, vanillic acid, coumaric acid, elagic acid, myricetin and juglone (Cosmulescu and Trandaflir, 2011). There are ample of evidences indicating that *Juglans regia* leaf possess potent antioxidant activity and it is anti-inflammatory, anti-carcinogenic and cardioprotective properties has been attributed to this (Erdemoglu et al., 2003; Almeida et al., 2008; Hosseinzadeh et al., 2011). In addition several animal studies have shown that, *Juglans regia* leaf extract is capable of ameliorating hyperglycemia and hyperlipidemia related to diabetes and even preventing toxic effects of streptozocin in rats (Asgary et al., 2008; Teymouri et al., 2010; Mohammadi et al., 2012). In the light of all of the above we felt a great need for a randomized double-blind, placebo controlled clinical trial study to evaluate the efficacy and safety of *Juglans regia* leaf extract in diabetic patients. The current study was design to investigate anti-hyperglycemic and anti-hyperlipidemic effects of the regia leaf extract in type II diabetic patients.

2. Materials and methods

2.1. Juglans regia leaves collection

*Juglans regia* leaves were collected at the end of May, from Institute of Medicinal Plants fields located in Karaj region of the Alborz province of Iran and identified by a botanist (Y. Ajani). Voucher specimens of the plant (number 2341) were preserved in the central herbarium of the Institute of Medicinal Plants. The leaves were washed and dried in shade at room temperature and ground to make soft powder.

2.2. Preparation of plant extract

The powder was extracted with 70% aqueous ethanol using percolation method at room temperature. The solvent was removed by filtering through Whatman no.1 filter paper and evaporating under reduced pressure at a maximum of 40 °C to give a crude extract. The extract was standardized through determining the total phenols content as described below.

2.3. Determination of total phenols

It has been well accepted that *Juglans regia* leaf extract is a strong scavenger of pro-oxidant reactive species due to presence of several phenolic component. Hence, concentration of total phenols is used as quality control for antioxidant properties of *Juglans regia* leaves (Amaral et al., 2004; Pereira et al., 2007). The concentration of total phenols in *Juglans regia* leaf extract was measured by the method described by Kim et al. (2003) with some modification (Kim et al., 2003). Briefly, an aliquot (1 ml) of the appropriately diluted extract or standard solutions of Gallic acid in water (50, 100, 150, 200 and 250 μg/ml) was added to a 25 ml volumetric flask containing 9 ml of distilled water. Reagent blank using distilled water was prepared. One milliliter of Folin and Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na2CO3 solution was added by shaking. The solution was then immediately diluted to volume (25 ml) with distilled water and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance versus prepared blank was read at 750 nm. Total phenols content of the extract was expressed as milligram Gallic acid equivalent per gram extract. Sample was analyzed in 3 replications.

2.4. Preparation of Juglans regia and placebo capsules

The *Juglans regia* and placebo capsules were prepared with identical appearance in the Research Institute of Medicinal Plants (Karaj, Iran). The *Juglans regia* capsules contained 100 mg of the *Juglans regia* leaves extract powder. Toast powder was chosen as the placebo and as the excipient in preparation of *Juglans regia* leaves extract powder.

2.5. Study protocol

The trial was planned according to a parallel group, double-blind and placebo controlled design which was performed in a Diabetes Care Clinic of Shariati hospital Tehran, Iran. The research protocol was carried out in accordance with the Declaration of Helsinki and subsequent revisions, and approved by the ethics committee at the Endocrinology and Metabolism Research Center of Tehran University of Medical Sciences. The patients were informed about the main rationale and aims of the study, and patient provided written informed consent for the participation.

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, measurements of vital signs, electrocardiogram, laboratory parameters, and finally a total of 61 volunteer patients (aged 40–65 years) with type II diabetes, diagnosed according to ADA criteria (American Diabetes association), were selected among 71 patients and enrolled in this study. The exclusion criteria for enrollment were insulin therapy, infectious disease, and pregnancy, receiving lipid-lowering drugs, corticosteroids, other soluble fiber treatment, lithium, carbamazepine, warfarin, digoxin or patients with clinically significant cardiovascular, renal, hepatic, gastrointestinal, pulmonary, and thyroid disease. The inclusion criteria for enrollment were type 2 diabetes with FBG between 150 and 200 mg/dl, HbA1c between 7% and 9%, aged between 40 and 60 years, more than 6 years duration of disease, taking no more than two 5 mg glitizamide and two 500 mg metformin tablets per day, and their diabetes were not controlled exclusively by diet. The patients were randomly divided in to two groups (placebo and *Juglans regia*) using a balanced randomization method. In *Juglans regia* group, 32 patients received100 mg *Juglans regia* leaf extract capsule two times a day, before meal for 3 months, and patients in placebo group (n=29) received placebo capsule with the same schedule. Selected 200 mg *Juglans regia* leaf extract was equal to 7 g dry *Juglans regia* leaves, the average doses (4–10 g ) prescribed daily by 10 herbalists in Tehran city, Iran. The patients were advised not to change their diabetic food regimen and anti-diabetic drugs dosage during the study. Patients were examined every month and the efficacy of treatment was checked by physician. Nursery staffs had a phone contact with subjects every day for the first week of the experiment, and every week.
from the second week to the end of study. Moreover, patients were asked to stay in contact with the department and let the nursery staffs know the details of any possible adverse effects related to the therapy.

2.6. Outcomes

FBG, Hba1c, insulin, c-peptide, lipid profile, body weight, Creatinine, BUN (blood urea nitrogen), ALP (alkaline phosphate), AST (aspartate transaminase) and ALT (alanine aminotransferase) were determined as main parameters of the study. Blood samples were collected after an overnight fasting (12 h). FBG, total cholesterol, HDL, LDL, and triglycerides levels were measured using Roche hitachi 717 clinical chemistry auto-analyzer and commercial kits (Pars Azmon Co., Iran). Moreover, serum insulin and c-peptide levels were measured by radioimmunoassay method (Immunotech, France).

All adverse events were recorded in each visit. To assess the possible adverse effects and tolerance, patients were given a detailed dictionary of standard terms diaries and asked to note incidence and severity of symptom and stayed in contact with the department. Finally before the final biochemical tests, when patients were blind of the results, they were asked to answer two 5-point rating scale questions ranging from 0 to 3. First was about the effectiveness and general satisfaction with treatment (0: none; 1: mild; 2: moderate; 3: potent; 4: very potent), and the second was about general complain (0: absent; 1: mild; 2: moderate; 3: severe; 4: very severe) (Matthys et al., 2003).

2.7. Statistical analysis

Statistical analyses were performed using IBM SPSS software (v.20). Data are expressed as mean ± SD and median (interquartile range). Values within groups were compared by paired sample Student’s t-test or Wilcoxon Signed Rank test, and comparisons between groups were performed using Independent Sample Student’s t-test or Mann–Whitney U test after testing for normality. General satisfaction and complain with treatment were assessed by chi square tests. A value of P<0.05 was considered as statistically significant.

3. Results

3.1. Total phenols

*Juglans regia* leaf yielded 2.5% dried extract. The leaf extract possessed total phenolic content expressed as Gallic acid equivalent 41 ± 4.4 mg/g of the dried extract.

3.2. Demographic information at baseline

Demographic characteristics of both groups, recorded at the beginning of study are summarized in Table 1. All 61 patients completed the study and there were no dropouts, and they did not modify their diets and drug regimen during the study. No significant differences in the baseline parameters were detected between two groups (P>0.05 using Independent Sample Student’s t-test) (Table 1).

<table>
<thead>
<tr>
<th>Placebo (n=29)</th>
<th><em>Juglans regia</em> (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>55.4 ± 0.70</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>13M/16F</td>
</tr>
<tr>
<td>Duration of disease (year)</td>
<td>5.6 ± 0.32</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.8 ± 0.92</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27.3 ± 0.38</td>
</tr>
<tr>
<td>Metformine (mg/day)</td>
<td>830 ± 538.51</td>
</tr>
<tr>
<td>Glibenclamide (mg/day)</td>
<td>8.3 ± 9.2</td>
</tr>
</tbody>
</table>

3.3. The effects of *Juglans regia* extract on FBG, Hba1c, insulin and c-peptide

There were no significant difference in average FBG and Hba1c measured at the beginning of study, between two groups (P>0.05; independent samples Student’s t-test). Table 2 shows the extract administration for 3 months significantly decreased FBG in *Juglans regia* group compared with the baseline (P<0.05; using pair sample Student’s t-test). The same results were detected in the level of Hba1c in *Juglans regia* group (P<0.05; using pair sample Student’s t-test). At the end of study, a comparison between two groups has shown that patients treated with the extract have lower level of FBG and Hba1c (P<0.05; using independent samples Student’s t-test). There was no significant difference in insulin or c-peptide level either within (P>0.05; using Wilcoxon Signed Rank test) or between groups (Fig. 1, P>0.05; using Mann–Whitney U test).

3.4. The effects of *Juglans regia* extract on lipid profile

A significant decrease in the average fasting blood levels of total cholesterol and triglyceride was observed in *Juglans regia* group compared with the baseline (Table 2, P<0.05; using pair sample Student’s t-test). Although not significant, the average total cholest erol level in the placebo group increased from 179.79 ± 9.0 mg/dL to 182.34 ± 9.01 mg/dL, and triglyceride from 158.44 ± 11.9 to 164.62 ± 18.4 after 3 month of placebo treatment (P>0.05; using pair sample Student’s t-test). The average LDL level in the *Juglans regia* group at the beginning of the study was 105.53 ± 5.7 mg/dL which decreased insignificantly to 98.58 ± 6.0 mg/dL after 3 month of *Juglans regia* treatment (P>0.05; using pair sample Student’s t-test).The level of HDL did not significantly change after 3 months of *Juglans regia* or placebo treatment (Table 2, P<0.05).

3.5. The effects of *Juglans regia* extract on other parameters

There were no significant changes in the level of Creatinine, BUN, ALP, AST and ALT within or between groups (P>0.05) at the end of study.

3.6. Satisfaction of patients from *Juglans regia* therapy

According to the entries of the patient diaries and their responses to the question, related to their general satisfaction with the treatment, patient in *Juglans regia* group were satisfied with *Juglans regia* treatment compared with the patients in the placebo group (Table 3, P<.0001; using chi-square test).

3.7. Tolerance

Adverse effect occurred at a similar overall incidence in both placebo and *Juglans regia* groups. No differences in body weight and systolic and diastolic blood pressure were observed within or
The fasting blood parameters before and after intervention in placebo and juglans regia treated groups. Values are expressed as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>After</th>
<th>(P_{\text{value}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>168 ± 70.54</td>
<td>170 ± 60.85</td>
<td>0.923</td>
</tr>
<tr>
<td>HbA1c (percent)</td>
<td>8.2 ± 1.72</td>
<td>7.89 ± 1.51</td>
<td>0.201</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>176.79 ± 48.47</td>
<td>182.34 ± 48.52</td>
<td>0.13</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>143.44 ± 64.08</td>
<td>164.62 ± 99.09</td>
<td>0.499</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>42.93 ± 9.15</td>
<td>42.96 ± 9.15</td>
<td>0.985</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>95.03 ± 32.85</td>
<td>97.72 ± 34.47</td>
<td>0.595</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.99 ± 0.22</td>
<td>0.91 ± 0.16</td>
<td>0.488</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>15.8 ± 4.85</td>
<td>16.9 ± 5.36</td>
<td>0.857</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>160.7 ± 56.01</td>
<td>152.0 ± 44.16</td>
<td>0.532</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>13.83 ± 4.20</td>
<td>12.21 ± 4.52</td>
<td>0.551</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>6.79 ± 3.12</td>
<td>6.5 ± 2.80</td>
<td>0.814</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>(P_{\text{value}})</td>
</tr>
<tr>
<td></td>
<td>165 ± 53.74</td>
<td>143 ± 65.05</td>
<td>0.033*</td>
</tr>
</tbody>
</table>

\* \(P < 0.05\), compared with the baseline.
\* \(P < 0.05\) compared with placebo group (using independent sample Student’s t-test).

Fig. 1. Box plot of insulin and C-peptide level (before intervention – after intervention) of juglans regia and placebo groups. (\(P > 0.05\), using Wilcoxon Signed Rank and Mann–Whitney U tests respectively) Values are expressed as median (interquartile range).

4. Discussion

The Juglans regia L. leaf has been traditionally used for treatment of diabetes mellitus in Iran, but to our knowledge no clinical studies have been conducted to determine its efficacy and safety in diabetic patients. In the present clinical trial, we have confirmed anti-hyperglycemic and hypolipidemic effects of Juglans regia leaf extract in patients with type II diabetes. Juglans regia leaf extract 200 mg daily treatment to diabetic patients reduced FBG, HbA1c as well as cholesterol and triglyceride levels after 3 months of the study. No significant change has been observed in the level of LDL and HDL. Majority of patients treated with Juglans regia leaf extract were satisfied with the new treatment and absence of moderate or serious side effects of the extract therapy except transient gastro intestinal upset at the first week of study had already been observed in outcomes of the study. In the mean time, our findings clearly show that, there were no significant changes in some biochemical markers of toxicity (ALT, AST, ALP and Creatinine).

Although several experimental studies confirmed anti-diabetic effect of Juglans regia leaf extract (Jelodar et al., 2008; Mohammadi et al., 2012) but still exact mechanisms for its hypoglycemic and hypolipidemic properties is not well defined. It seems that anti-oxidant components present in Juglans regia extract play an important role (Maxwell et al., 1997; Evans et al., 2002). Its potent anti-oxidants property is even comparable to the reference standards (ascorbic acid and butylated hydroxytoluene, BHT) at the same dose (Pereira et al., 2008; Rather et al., 2012). Chemical compositions of Juglans regia leaf extract are well characterized.
It has been shown that, leaves are rich of phenolic acids and flavonoids including 3- and 5-cafeoylquinic acids, quercetin-3-galactoside, quercetin 3-arabinoside (Amaral et al., 2004; Pereira et al., 2007; Yoo et al., 2008; Jalili and Sadeghzadeh, 2012) with anti-diabetic property (Narita and Inouye, 2009; Kim et al., 2011). In fact, researchers have reported antioxidant activity of phenolic compounds (Sun et al., 2002; Silva et al., 2004; Carvalho et al., 2010) and their favorable effects in management of hyperglycemia in diabetic patients (Rahimi et al., 2005; Xiao et al., 2013).

Another proposed anti-diabetic mechanism of Juglans regia leaf extract is its favorable effects on beta cells regeneration as reported in diabetic rats (Jelodar et al., 2008). In addition one more possible mechanism for anti-diabetic effects of the extract could be through its anti-inflammatory properties, since inflammation and activated innate immunity are an important factor in the pathogenesis of type 2 diabetes (Pickup, 2004; Kolb and Mandrup-Poulsen, 2005; Hosseinzadeh et al., 2011). Of note, the main limitations of the current study are small sample size, short duration of therapy and lack of determination of chemical composition of the Juglans regia leaf extract, due to lack of funding and equipment. In conclusion, the results suggest that 100 mg/kg of Juglans regia leaf extract two times daily for 3 months can significantly decrease FBG, Hba1c, triglyceride and total cholesterol of type II diabetic patients without important adverse effects. More studies are required to determine long-term efficacy and safety of Juglans regia leaf extract.

Acknowledgments

The collaboration of the Institute of Medical Plants and the medical and nursing staff of the Diabetic Center of Shariati Hospital in expert technical assistance are gratefully acknowledged by the authors. This study was supported by a research Grant number 257/2011 from the Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran.

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