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Original Article

Extracts of the walnut leaf (*Juglans regia L.*) improved activity of sorbitol dehydrogenase in diabetic male rats

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Abstract

Introduction: Walnut leaf contains large amounts of antioxidants, phenolic compounds and flavonoids and the beneficial effect of these compounds in the treatment of diabetes has been shown. This study investigated the effect of cyclohexane and ethanol extract of walnut leaf on activity of sorbitol dehydrogenase (SDH) enzyme.

Methods: Fifty- six adult male Sprague-Dawley rats were divided into seven groups and treated for 30 days as follows: control and diabetic control (received sesame oil as vehicle), control cyclohexane & control ethanol (received 250 mg/kg body weight of cyclohexane and ethanol extracts respectively), diabetic cyclohexane 250 (received 250 mg/kg cyclohexane extract), and diabetic ethanol 150 & diabetic ethanol 250 (received 150 and 250 mg/kg ethanol extract respectively). Diabetes was induced by single injection of streptozotocin (60 mg/kg, ip). Body weight and blood glucose were recorded weekly and in the last day of treatment animals were sacrificed by whole blood collection directly from the heart. Activity of SDH was measured in the serum by ELISA method.

Results: Oral administration of cyclohexane extract of walnut leaf at a dose of 250 mg/kg in the diabetic group improved blood glucose significantly compared to other diabetic groups. Administration of both extracts reduced activity of SDH compared to diabetic control group significantly. There was no significant difference of body weight between treatment groups and diabetic control group at the end of the treatment period.

Conclusion: Cyclohexane extract of walnut leaf decreased blood glucose significantly, while both extracts reduced activity of SDH significantly in diabetic animals.

Keywords:
Diabetes mellitus;
Sorbitol dehydrogenase;
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Introduction

Diabetes mellitus as one of the most important endocrine and metabolic disorders, affects a large population of our community. Most diabetic patients suffer from its long-term complications such as neuropathy, nephropathy, retinopathy, cataracts and heart attack. These complications result from chronic hyperglycemia and damage blood vessel walls and peripheral nerves. One of the important mechanisms which was proposed be involved in pathogenesis of diabetic complications is activation of polyol pathway (Oates, 2008). In diabetic patients, through polyol pathway excess glucose is reduced to sorbitol and then to fructose. One of the two key enzymes in

polyol pathway is sorbitol dehydrogenase (SDH) which converts sorbitol into fructose in the presence of nicotine amid adenine dinucleotide (NAD^+) as an electron acceptor. Fructose and its metabolites such as fructose-6-phosphate and triose phosphate can be glycosylated and start oxidative stress (Takagi et al., 1995). The existing evidence imply that walnut leaf (*Juglans regia L.*) has strong antioxidant, anti-inflammatory, anti-cancer and heart-protective properties (Erdemoglu et al., 2003; Almeida et al., 2008; Hosseinzadeh et al., 2011; Zheng and Wang, 2001). Studies have shown that the use of walnut leaves in the form of aqueous-alcoholic extract, alcoholic, cyclohexane and powder decrease blood glucose level in alloxan or streptozotocin (STZ)-induced diabetic rats (Jelodar and Nazifi, 2001; Asgary et al., 2008; Divband et al., 2010). Dietary intake of cyclohexane, ether and ethanol extracts of walnut leaves was reported to decrease the concentration of glucose, cholesterol, triglyceride and serum urea nitrogen (Jelodar and Nazifi, 2001). Polar (ethanol) and non-polar (cyclohexane) solvents may release different effective materials which are found in walnut leaf; hence this study was conducted to investigate the effect of oral administration of different doses of cyclohexane and ethanol extract of walnut leaf on body weight, blood glucose and sorbitol dehydrogenase activity in normal and diabetic rats.

Materials and methods

Preparation of cyclohexane and ethanol extract of walnut leaf

Walnut leaves were collected from the walnut farm of Agriculture College of Shiraz University, Shiraz, Iran. The leaves were dried in the shade (22±2 °C) for 4-5 days; dried leaves were ground into a fine powder using a homogenizer. The cyclohexane extract of walnut leaves was obtained according to a previous report (Jelodar and Nazifi, 2001). Briefly, dried leaves were milled and then soaked in cyclohexane for 24 hours, after filtration, the solvent was removed and the extract lyophilized. Ethanol extract of walnut leaf was prepared by the same method (Jelodar and Nazifi, 2001)

Animals

Fifty-six male Sprague-Dawley rats, weighing 200±20 g, were purchased from Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences. Animals were kept at temperature (22±2 °C), relative humidity (38 %), and 12/12 h light/dark cycle and had free access to food and water *ad libitum.*

Animal ethics

This study was accomplished under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran. Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes were followed.

Study design

The rats were allocated into seven groups comprising eight animals in each group as follows:

Normal control: Healthy rats that received 0.1 M citrate buffer (pH= 4.5) at the begining of the study and received only the vehicle (sesame oil) by gavage for 30 days.

Control cyclohexane 250: Healthy rats that received 0.1 M citrate buffer (pH= 4.5) at the begining of the study and were treated with cyclohexane extract of walnut leaf dissolved in sesame oil at a dose of 250 mg/kg /day by gavage for 30 days.

Control ethanol 250: Healthy rats that received 0.1 M citrate buffer (pH= 4.5) at the begining of the study and were treated with dissolved ethanol extract of walnut leaf at the dose of 250 mg/kg /day in sesame oil by gavage for 30 days.

Diabetic control: Diabetic rats that were treated only with vehicleby gavage for 30 days.

Diabetic cyclohexane 250: Diabetic rats treated with cyclohexane extract of walnut leaf dissolved in sesame oil at the dose of 250 mg/kg /day by gavage for 30 days.

Diabetic ethanol 150: Diabetic rats treated with ethanol extract of walnut leaf dissolved in sesame oil at the dose of 150 mg/kg /day by gavage for 30 days.

Diabetic ethanol 250: Diabetic rats treated with ethanol extract of walnut leaf dissolved in sesame oil at the dose of 250 mg/kg /day by gavage for 30 days.

In the diabetic and treatments groups, diabetes was induced by single injection of streptozotocin (Sigma,USA). For this purpose, the animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared

solution of STZ (60 mg/kg) in 0.1 M citrate buffer (pH=4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. If 72 h after injection of STZ, fasting blood glucose was more than 250 mg/dl, animal was selected and used for this study (Mazloomi et al., 2010). In the last day of treatment, blood samples were collected directly by heart puncture; serum was isolated and stored at -80 °C for further studies.

Blood glucose assay

Fasting blood glucose levels were measured at 0th, 7th, 14th, 21st and 30th day using glucometer (Accu-CHEK ACTIVE, Roche, Germany).

Sorbitol dehydrogenase assay

Sorbitol dehydrogenase was measured using a solid phase sandwich ELISA method (Rat ELISA Kit; Bioassay Technology Laboratory, Shanghai Crystal Day Biotech Company, LTC, China).

Statistical analysis

Results are reported as Mean±SEM. All data were performed with ANOVA followed by post Hoc multiple comparisons and Duncan multiple range test. $P<0.05$ was taken to indicate a significant difference between groups.

Results

Comparing of body weight

Changes of body weight of all study groups during treatment are presented in the Table 1. There was no significant difference between control and treatment control (ethanol and cyclohexane) groups, while in all diabetic groups weight loss was significant compared to non-diabetic groups. There was no significant difference between diabetic groups.

Blood glucose level

Comparing of blood glucose among study groups shows no significant difference between non-diabetic groups; however in diabetic groups consumption of cyclohexane extract significantly improved blood glucose compared to other diabetics (Table 2).

Activity of sorbitol dehydrogenase

Activity of sorbitol dehydrogenase (Table 3 and Fig.1) in diabetic control group was higher than other groups, but treatment with both extracts could decrease activity of this enzyme significantly compared to diabetic and non-diabetic control groups ($P<0.05$).

Table 1: Body weight (g) changes in different groups during 30 days of experimental period (Mean ±SEM).

Groups	Day 0	Day 7	Day 14	Day 21	Day 30	% of changes BW
Normal control	227.75±3.13 Aab	253.37±3.43 Bb	265.12±4.76 Cc	279.75±5.00 Dd	285.25±5.61 Eb	+25.24
Control cyclohexane 250	224.12±3.94 Aab	235.50±4.45 Bab	248.12±4.79 Cbc	265.87±5.54 Dcd	276.87±6.37 Eb	+23.53
Control ethanol 250	221.37±4.51 Aab	233.12±6.05 Bab	244.12±6.39 Cabc	261.75±7.49 Dbcd	273.75±8.96 Eb	+23.66
Diabetic control	213.00±11.47 Aa	222.75±9.98 Aa	202.12±9.80 Aa	229.62±15.35 Aab	212.75±14.60 Aa	−0.11
Diabetic cyclohexane 250	227.25±4.76 Aab	233.25±7.20 Aab	236.75±10.56 Aabc	239.87±14.36 Aabc	225.62±17.30 Aa	−0.71
Diabetic ethanol 150	228.25±5.97 ABCab	214.87±7.04 ABCa	217.62±8.52 ABCab	212.25±10.92 Ba	198.87±12.50 Ca	−12.87
Diabetic ethanol 250	235.75±4.77 Ab	231.25±9.81 Aab	255.00±31.63 Abc	213.62±16.22 Aa	206.00±17.82 Aa	−12.61

Different small alphabetic letters show significant differences between groups.

Different capital alphabetic letters show significant differences within group during experimental period.

Table 2: Changes of fasting blood glucose (mg/dl) concentrations in different groups during 30 days of experimental period (Mean ±SEM).

Groups	Day 0	Day 7	Day 14	Day 21	Day 30
Normal control	111.00±2.53 ABa	104.12±3.40 ABCa	101.50±2.59 ABCa	101.50±2.55 ABCa	97.75±1.41 Ca
Control cyclohexane 250	101.12±2.85 Aa	82.25±2.49 Ba	80.00±2.61 BCa	81.87±2.96 BCDa	88.12±3.44 BDEa
Control ethanol 250	104.37±3.09 Aa	85.37±2.17 Ba	79.62±1.97 BCa	84.37±1.90 BDa	90.50±3.62 ABDa
Diabetic control	454.37±53.37 Ac	390.37±32.91 Ac	336.12±17.02 Abc	366.25±31.47 Ac	367.25±44.85 Ac
Diabetic cyclohexane 250	349.12±45.08 Ab	245.75±53.85 ABCb	281.00±59.78 ABCb	244.62±54.12 BCb	198.62±38.51 Cb
Diabetic ethanol 150	422.00±25.75 ABCDbc	306.50±34.96 Abc	394.75±43.52 BCc	437.25±50.57 BCc	450.00±51.99 BDc
Diabetic ethanol 250	349.00±34.85 Ab	333.25±33.41 Abc	386.62±48.29 Ac	449.12±50.46 Ac	448.37±50.51 Ac

Different small alphabetic letters show significant differences between groups.
Different capital alphabetic letters show significant differences within group during experimental period.

Table 3: Changes of sorbitol dehydrogenase (IU/L) activities in different groups during 30 days of experimental period (Mean ±SEM).

Group	Normal control	Control cyclohexane 250	Control ethanol 250	Diabetic control	Diabetic cyclohexane 250	Diabetic ethanol 150	Diabetic ethanol 250
SDH	57.39±1.36 b	56.03±2.04 b	59.09±2.72 b	114.10±3.73 c	47.20±1.70 a	44.83±1.02 a	43.47±1.70 a

Different small alphabetic letters show significant differences between groups.

Discussion

In all diabetic groups weight loss was significant compared with non-diabetics, which is in agreement with other reports (Mazloomi et al., 2010). Oral administration of both extracts did not have a significant effect on body weight of diabetic animals, which could be due to hyperglycemic condition of animals in spite of somehow improvement in their statues. Similar results were reported following administration of plants with hypoglycemic effects (Mohammadi et al., 2011; Islam, 2011; Torrico et al., 2007). Weight loss in diabetics may result from destruction or decomposition of protein structure (Rajkumar et al., 1991), insufficient dose of extract or hyperglycemic condition of diabetic groups even after treatment. In the treated group, cyclohexane extract of walnut leaf significantly improved blood glucose compared to diabetic control group which supports previous reports (Jelodar and Nazifi, 2001; Divband et al., 2010). Walnut leaf and ridge extracts are known to contain high amounts of strong antioxidant components like vitamin C, vitamin E, beta-carotene, lipoic acid, quercetin, naphtoquinones, flavonoids, gallic acid, polyphenols, linoleic and linolenic acids, tannins and folates that have been shown to be very beneficial anti-diabetic effects in *in vitro* or *in vivo* models and in humans (Haak et al., 2000; Tapsell et al., 2004). Walnut leaf is also rich in phenolic acids and flavonoids (Jalili and Sadeghzade, 2012) and phenolic compounds have antioxidant activity (Carvalho et al. 2010). Favorable effects of phenolic compounds (Carvalho et al., 2010; Xiao et al., 2013)

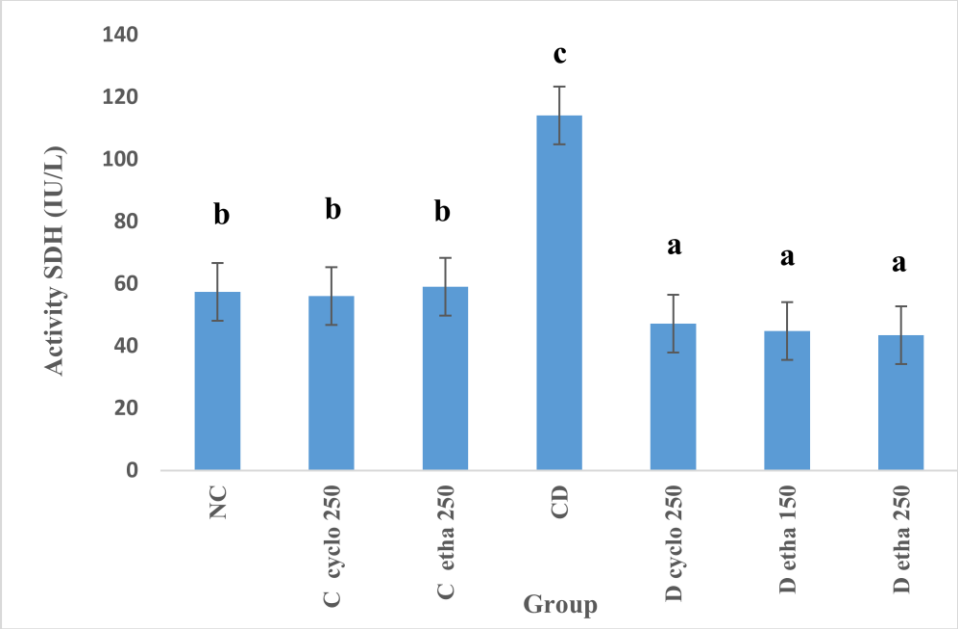

Fig.1. Comparison of sorbitol dehydrogenase activity in different groups during 30 days of experimental period (Mean ±SEM).
Different small alphabetic letters show significant differences between groups.
NC: Normal control**, C cyclo 250:** Control cyclohexane 250**, C etha 250:** Control ethanol 250**,**
CD: Diabetic control**, D cyclo 250:** Diabetic cyclohexane 250**, D etha 150:** Diabetic ethanol 150**,**
D etha 250: Diabetic ethanol 250

and flavonoids (Vaya and Aviram, 2001) in controlling hyperglycemia have also been reported. Hypoglycemic activity of walnut leaf may be mediated by decreasing glucose 6-phosphatase activity (a liver enzyme which increases its activity in diabetes mellitus) (Pushparaj et al., 2007). This enzyme has an important role in regulation of blood sugar and liver glucose output, and thus results in blood glucose reduction (Dhandapani et al., 2002). Other possible mechanisms underlying the glucose lowering effect of *J. regia* might be due to increased release of insulin from remnant β-cells and/or regenerated β-cells, restored insulin sensitivity (Jelodar et al., 2007), interference with the absorption of dietary carbohydrates in the small intestine (Ortiz-Andrade et al, 2007) and utilization of glucose by peripheral tissues mediated by an insulin dependent glucose transporter is facilitated (Del Rio et al., 2013). Regenerative effect of walnut leaf extracts on beta cells in pancreas of diabetic rats has already been reported as a possible mechanism of hypoglycemic effect of walnut leaf (Jelodar et al., 2007). In general, given the above mentioned studies, it can be said that anti-hyperglycemic and hypo-glycemic effects of walnut leaf extract is probably due to flavonoids, such as quercetin and kaempferol. Their possible mechanisms can be associated with anti-diabetic effect (Abdelmoaty et al., 2010; EL Naggar et al., 2005; Wolff, 1993), anti-oxidant effect (Akiyama et al., 2001; Mahesh and Menon, 2004; EL Naggar et al., 2005; Wolff, 1993), impact on hepatic glucokinase (Hii et al, 1985),inhibition of gastrointestinal absorption of glucose, glucosuric effect (Lukacinova et al., 2008) and/or insulin-like external pancreatic mechanisms (Sokeng et al., 2007; Zanatta et al., 2008).

Administration of walnut leaf to diabetic groups decreased SDH activity. SDH catalyzes conversion of sorbitol to fructose in the presence of NAD. It has been reported that SDH activity increases in diabetic rats, leading to the increase of available fructose which is a 10-fold better substrate than glucose for glycosylation (Brownlee, 1992). Since plasma and liver glucose concentrations are increased in diabetic rats, more glucose is converted to sorbitol. The elevation of SDH activity observed in diabetic rats could be due to increased availability of sorbitol (Latha and Pari., 2004). Fructose generated by the polyol pathway can be metabolized into fructose-3-phosphate by 3-phosphokinase which is converted to 3-deoxyglucosone as major precursor in the formation of advanced glycation end-products (Yan et

al., 2003).

Fructose and its metabolites, such as fructose-6-phosphate and triose-phosphate, can be triggers of glycation and oxidative stress (Takagi et al., 1995). Decrease activity of SDH in diabetic treated group could be due to hypoglycemic effect of walnut leaf extract or its direct effect on activity of this enzyme, as it have been reported for some other plants (Latha and Pari, 2004). For example ethanolic extract of the root bark of Cananga odorata (Lam) (Anitha and Indira, 2006), Scoparia dulcis extract (Latha and Pari, 2004) and quercetin (Abd el-baky, 2011) was reported to reduce SDH.

Conclusion

In summary, oral administration of cyclohexane extract of walnut leaf decreased glucose significantly and this extract at the dose of 250 mg/kg and ethanol extract of walnut leaf with both used doses caused significant reduced activity of sorbitol dehydrogenase in all diabetic treated groups compared to control groups (normal, diabetic and treatments).

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Conflict of interest

The authors declare no conflicts of interest.

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