Beneficial effects of walnut shell extract on glucose and lipids profile in diabetic rats compared with Glibenclamide

Roya Mirbadalzadeh¹ and Zahra Shirdel²
¹Department of Biology, Ardabil Payame Noor University, Iran
²Department of Biology, Bojnourd Payame Noor University, Iran.

ABSTRACT
Diabetes mellitus is the most prevalent endocrine disease result in blood glucose increment, carbohydrate, lipids and protein metabolism disorders. Primary and effective cure for diabetes is insulin and hypoglycemic drugs usage, but these compositions have some undesirable side effects. Herbal medicine is the oldest kind of diseases cure has recognized. However, rational prescription of effective medicinal plants for diabetes cure requires precise information of action mechanism of these plants. In present study, diabetes induced in rats, and then hypoglycemic effect of walnut husk hydroalcoholic extract and blood lipoproteins (LDL, HDL, VLDL), triglyceride and total cholesterol changes were evaluated by enzymatic kits. The results showed significant reduction of glucose, triglyceride, VLDL and LDL levels in extract group in comparison with diabetic control (P=0.001). Glucose and LDL reduction by walnut shell are similar to glibenclamide effect. And TG, VLDL reduction by walnut are more than glibenclamide effect. Walnut also could increase HDL levels significantly in comparison with diabetic control (P=0.001) that this effect is similar to glibenclamide effect on HDL. In summary, the positive effects of walnut green husk suggest a possible role of this plant in improving glucose and lipid metabolism in diabetics.

INTRODUCTION
Diabetes mellitus is the most prevalent endocrine disease results in blood glucose increment, carbohydrate, lipids and protein metabolism disorders. This disease is created as a result of glucose cell dissabsorption derives from decrease of insulin secretion or insulin resistance of body cells and result in intracellular metabolism changes in most tissues such as liver[1]. Considering side effects of diabetes in patients, investigation of its cure and prevention ways are necessary. Though, primary and effective cure for diabetes is insulin and hypoglycemic drugs usage, but these compositions have some undesirable side effects. Medicinal plants and their derivatives have been used to cure diabetes since past years but scientific investigations are necessary to prove of their effects [2].

Walnuts (Juglans regia) are plants in the family Juglandaceae. Walnut fruit is drupe includes bitter materials. Walnut is a useful tree in nurture usages and traditional medicine, and its remedy properties have been recognized since past years. Useful parts of walnut tree are leaf, second shell and fleshy part of green fruit and its wood. Green husk of walnuts fruit called epicarp. Epicarp has effects similarly walnut leaf and includes: emulsion, glucose and organic acids such as citric acid, malic acid, phosphates, oxalate calcium. Other materials in its green husk are Staresinolic acid, betulinic acid, daucosterin, 4,5-O-isopropylidene-α-tetralone, 4-methoxy-α-tetralone-5-Oa-glucopyranoside, 4-ethoxy-8-hydroxy-α-tetralone-2,3-dihydroxy-1-(4-hydroxy-phenyl)-propan-1-one, dihydrophaseic acid. Juglon is 5-Hydroxy 1,4 naphthoquinone that there is only in green and fresh parts of walnut and it is one of the most important flavonoids of walnuts green husk [3,4,5,6]. Walnut leaf and shell have some medicinal effects, as walnut green husk has antioxidant [7,8] antifungal [9,10], astringent, wart liquidator effects and uses for skin diseases and anemia cures.

Walnut leaf and unripe fruits fleshy part is a bitter reinforcer and has worm rebuff, anti-diabetic, anti-phthisis effects [11]. In present study, we induced diabetes in rats. After diabetes verification, we evaluated effect of Juglans regia shell hydroalcoholic extract on blood glucose.

MATERIALS AND METHODS
Plant materials and extraction. Fresh husk of J. regia were bought from Ardabil Department for Natural Resources (1 kg), and authenticated by Dr. Mohammad Ebrahimzade, Department of Biology, University of Esfahan, Iran. A specimen voucher (AS-AP-04-06-28) was deposited at the herbarium located at the Department of Biology, University of Ardabil. The husk was cleaned and powder was prepared with mill, and ethanol 96% was added to cover the surface of the powder. Then it was positioned on the shaker. After 24 hours the solution was filtered through filter paper (Whatman qualitative grade 1), and again ethanol 75% was added to the remained waste, and was positioned on the shaker for 12 hours. Finally, the combined filtrate was then concentrated in a rotary evaporator (35–40 °C), to a thick, dark green colored crude extract up to 1/6 the primitive volume. For proteins isolation and material refining, after the filtered solution decantation 3 times by chloroform, was positioned in incubator at 50 °C. After a few days, the powder was ready and included net and effective material of the plant. A crude residue (40g) was obtained giving a yield of 4 %. The powder was dissolved in normal saline for experiments, and dilutions were made fresh on the day of experiment.

Animals: The experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on
Life Sciences, National Research Council (NRC, 1996). Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC). Male rats (Rattus Norvegicus Allivias) used in the study (190-220 g) were housed in the animal house of the Ardabil Payame Noor University. Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature (23-25°C), relative humidity (45-55%) and 12 hrs dark/light cycles were maintained in the quarantine. All the animals were fed with rodent pellet diet and water was allowed ad-libitum under strict hygienic conditions. After the adaptation period, each group of rats was weighted and marked, and then treated by the specified dose of materials.

**Diabetes induction.** Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetron) is an oxygenated pyrimidine derivative [12]. Glucose and alloxan structural similarity causes alloxan connects and enters beta cells. Alloxan degenerates specially beta cells thus uses as a suitable material to induce diabetes in animals. Meanwhile alloxan causes Reactive Oxygen Species production only in Langerhauns islets [13]. Alloxan injection causes diabetes induction in rats which it’s similar to human type 1 diabetes. In this study, criterion for diabetes induction was blood glucose more than 300mg/dl. After 72 hours of alloxan injection, the diabetic rats were separated and used for the study. Animals were assigned to 4 groups having the following characteristics:

1) Normal group: was treated by saline (2 ml/kg, i.p.)
2) Diabetic control group: was treated by alloxan monohydrate (120mg/kg, i.p.) for 3 days alternately. Then, blood glucose was evaluated by blood glucose test meter (Glutest PRO R; Sanwa-kagaku, Nagoya, Japan).
3) Extract group: was treated by alloxan monohydrate for 3 days alternately and, after blood glucose evaluation and diabetes verification, animals received hydro-alcoholic extract of *J. regia* (100 mg/kg, i.p.) for 10 days alternately.
4) Glibenclamide group: was treated by alloxan monohydrate for 3 days alternately and diabetes verified after blood glucose evaluation, and after 48 hours, received also glibenclamide (500mcg/kg/i.p) for 10 days alternately. 72 hours after extract administration, the animals were anesthetized and blood samples were collected from heart of each rat and were analyzed for glucose and lipid content by enzymatic kits.

**Statistical analysis.** All the experiments were repeated at least 3 times with appropriate controls. Data are presented as the Mean±SD and P<0.05 was considered statistically significant. Statistical analysis was performed using a one-way ANOVA and relevant figures were drawn with Excel.

**Results**

The results of glucose, triglyceride and lipoproteins biochemical experiments mentioned table1.

**Glucose** mean difference of extract group with normal and control groups is significant(p<0.05)

**Triglyceride** mean difference of extract group with control group is significant(p<0.05).

There is not significant mean difference between the groups for cholesterol due to regulator mechanisms of plasma cholesterol concentrations.

**LDL** mean difference of extract group with control group is significant(p<0.05)

**HDL** mean difference of extract group with control group is significant(p<0.05)

**VLDL** mean difference of extract group with control group is significant(p<0.05) Each column illustrates mean±SD

According to fig.1,2,3,4, the extract has significantly reduced glucose, triglyceride, LDL and VLDL level compared with control group, and considering to there is no mean difference between extract group and glibenclamide group, thus *J.regia* could reduce mentioned factors as glibenclamide.

![Figures 1, 2, 3, 4](image)

**Fig.5** displays that the extracts have increased significantly HDL level in diabetic rats. There is no significant mean difference between the extract and glibenclamide group(p>0.05) thus the extract increased HDL level as glibenclamide.

**Figures 5, 6, 7, 8**

**Discussion**

In present study hypoglycemic effect of *J.regia* husk hydroalcoholic extract was evaluated in diabetes-induced rats and results compared with glibenclamide effect. Glibenclamide is one of the sulfonylurea antidiabetic drugs which increases insulin secretion of beta cells. In addition to, this drug has insulin-like effects on glucose metabolism, as decreases glycogenesis and gluconeogenesis, thus by reduction of two mentioned mechanisms, blood glucose is reduced [14, 15]. Investigations have been shown alloxan special toxicity for beta cells is due to fast cell absorb by pancreas beta cells and free radicals production by alloxan. Free radicals damage proteins, lipids, carbohydrates, nucleic acids, etc…. herewith affect cell activity such as membrane function, metabolism and gene expression, as some cells lose their structures and functions. According to the studies, oxidative damage of free
radicals is chief reason of histological and cell damages in some diseases such as atherosclerosis, cancer, diabetes mellitus, etc... [16]. Anti-oxidants are compositions which guard cell membranes and different compositions of organism. Mechanism of anti-oxidant action is: free radicals agglomeration, electron transfer to these oxidants and inactivation of them [17, 18]. Anti-oxidant action is: free radicals agglomeration, electron transfer to these oxidants and inactivation of them [17, 18].

J. regia green shell includes anti-oxidants such as flavonoides. Juglon is most important flavonoide of walnut shell. Recent studies have been shown flavonoides reduce blood sugar[18,19]. Following alloxan injection, and blood sugar increasing, triglyceride increased too, demonstrates insulin role in lipids metabolism adjustment [20]. As alloxan induced diabetes mellitus in rats includes clear undesirable changes in plasma lipids and lipoproteins as in alloxan or streptozotocin-induced diabetic rats, increase triglycerides and cholesterol level [21, 22, 23]. On the other hand, in alloxan-induced diabetic rats, glucose increment result in cholesterol, triglyceride, LDL and VLDL increment and HDL reduction[24,25] which it’s partly justifier of undesirable changes in plasma lipids in the diabetic rats of this study.

Following glibenclamide injection, triglyceride decreased. In addition too, LDL decreased and HDL increased too, it’s similar to Braunner, Washort, Regitz and Tuval findings [26]. The extract reduced triglyceride, cholesterol, LDL and VLDL levels and increased HDL too. Considering to occurs stress oxidative intensification in diabetes mellitus and result in blood biochemical changes in diabetes type1[21], and walnut green shell decreases stress oxidative due to high level anti-oxidant materials such as flavonoides like juglon, thus result in desirable changes on glucose and triglyceride levels in rats. In addition to in diabetes type 1, reduced vascular lipoprotein lipase activity, thus maybe walnut effective materials can affect this enzyme action and return it to normal level [27, 28] as breakdown triglycerides in vessels, and triglycerides hydrolysis result in their reduction in plasma. By triglyceride reduction by the extract, VLDL level reduced significantly too. About this event we can say: intercellular triglyceride increasing causes VLDL synthesis increasing. Because triglyceride level was reduced significantly by the extract, it is safe to expect VLDL synthesis to reduce. Meanwhile, 90% blood VLDL made in liver and liver cells triglycerides enter VLDL structure, thus each factors reduce triglycerides, can decrease blood VLDL too[29]. Considering VLDL involves LDL particle generation indirectly, thus by significant reduction of VLDL by the extract, we can expect LDL levels to decrease too.

Considering plasma HDL concentration has inverse association with plasma triglyceride concentration, and recalling that J. regia shell could reduce triglyceride level, then by decrease of triglyceride level, increase of HDL level should be expected [29,30].

Conclusions

According to the results, defines J. regia shell has hypoglycemic effect in diabetes mellitus experience model in rat and it causes useful changes on blood lipids. We suggest more investigations to clear the extract mechanism on blood biochemical parameters in both normal and diabetic treatments.

Acknowledgments

This research was supported by a research grant (0102/32/2405) from Ardabil Payame Noor University, Republic of Iran (2009).

References

19- Zargari A. Medicinal plants. 4th volume, Tehran University publication; 1997. p325-328
23- Shirdel Z, Madani H, Mirbadalzadeh R. Investigation into the hypoglycemic effect of hydroalcoholic extract of Ziziphus Jujuba Leaves on blood glucose and lipids in Alloxan-Induced diabetes in rats. IJDLD. 2009; 7(3): 275-281

Table 1- Effect of J.regia shell extract on glucose, cholesterol, triglyceride and lipoproteins levels in rats

<table>
<thead>
<tr>
<th>Index</th>
<th>experimental groups (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>normal</td>
</tr>
<tr>
<td>glucose (mg/dl)</td>
<td>124.61±5.35</td>
</tr>
<tr>
<td>triglyceride (mg/dl)</td>
<td>106.30±4.42</td>
</tr>
<tr>
<td>cholesterol (mg/dl)</td>
<td>91.79±12.32</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>23.37±6.88</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>49.16±7.34</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>21.25±2.38</td>
</tr>
</tbody>
</table>