

Research Article

EVALUATION OF THE BLOOD-GLUCOSE REDUCING EFFECTS OF WALNUT GREEN HUSK EXTRACT IN DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death, and third when its fatal complications are taken in to account. Medicinal plants and their derivatives have been used to cure diabetes since past years but scientific investigations are necessary to prove of their effects. In present study, diabetes induced in rats, and then hypoglycemic effect of walnut husk hydroalcoholic extract was evaluated. The results showed the extract reduced significantly glucose. In summary, the positive effects of *J.regia* green husk suggest a possible role of this plant in improving glucose metabolism

Keywords: Blood Glucose, *J.regia*, Hypoglycemic, Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death, and third when its fatal complications are taken in to account¹. Considering side effects of diabetes in patients, investigation of its cure and prevention ways are necessary. Though, primary and effective cures for diabetes are 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^{2,3}. Probable efficiency of medicinal plants for cure of diabetes and their abundance in most place of the world, facilitate their usage.

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 Sioresinolic acid, betulinic acid, daucosterin, 4,5-O-isopropylidene- α -tetralone, 4-methoxy- α -tetralone-5-O- α -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 flavonoides of walnuts green husk⁴⁻⁷. Walnut leaf and shell have some medicinal effects, as walnut green husk has antioxidant^{8,9}

antifungal^{10,11}, 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 and blood cathartic effects and uses to cure of joints dropsy, chronic snivels, sores absterge and their redress¹². 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 coloured crude extract up to $\frac{1}{3}$ 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 cycle 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: For inducing diabetes in rats, we used alloxan monohydrate (Sigma Chemical Company, Germany) 120mg/kg (i.p.)¹ solved in saline. Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative¹³ and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig¹⁴. 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 Langerhans islets¹⁵. 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 3 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.

72 hours after extract administration, the animals were anesthetized and blood samples were collected from heart of each rat and were analyzed for glucose content by using glucose oxidase peroxidase (GOD-POD) method using a visible spectrophotometer at 505 nm.

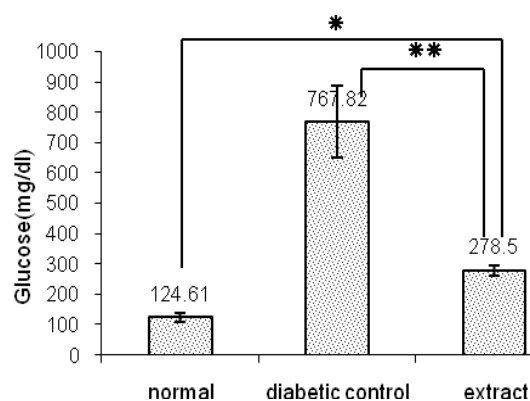
Statistical analysis: All the experiments were repeated at least 3 times with appropriate controls. Data are presented as the Mean±SD and P<0.05 was considered

statistically significant. Statistical analysis was performed using a one-way ANOVA and the relevant figures were drawn with Excel.

RESULTS AND DISCUSSION

According to fig.1, *J.regia* extract have been reduced significantly glucose level (p<0/001) from 767.82mg/dl in diabetic rats to 278.50mg/dl in extract group.

Figure 1: Glucose level of the extract group compared with other groups.



Data are presented as Mean±SD for 10 samples *p<0.01, **p<0.001

Investigations have been shown alloxan special toxicity for beta cells is due to fast cell absorption 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...¹⁶⁻¹⁹. 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^{20,21}. *J.regia* green husk includes anti-oxidants such as flavonoides. Juglon is most important flavonoid of walnut shell²². Recent studies have been shown flavonoids reduce blood sugar²⁰.

Other mechanism of walnut shell hypoglycemic activity is: Walnut effective materials decreases glucose 6-phosphatase activity (a liver enzyme which increased its activity in diabetes mellitus)²³. This enzyme has important role in regulation of blood sugar and liver glucose output, thus result in blood glucose reduction²⁴. One of the extract possible effects is liver phosphorylase inhibition, which inhibits glycogen storage breakdown in hepatic cells and increases the enzyme activity that result in glycogen synthesis improvement.

CONCLUSION

According to the results, defines *J.regia* green husk 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. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

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