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THE INFLUENCE OF AQUEOUS EXTRACT OF WALNUT ON REDUCING BLOOD GLUCOSE LEVELS IN RATS

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ABSTRACT

Diabetes causes hormonal, biochemical changes and metabolic damages on body. Therefore, in this research, therapeutic effects of the liquid extract of walnut on Streptozotocin-induced diabetic rats have been discussed. 30 adult male Westar rats were classified into five six-member categories: the control group subjected to simultaneous injection of STZ and citrate buffer. The first experimental group that became diabetic through the injection of 55mg/kg of STZ. The second, third and fourth experimental groups including six diabetic rats that were treated by daily gavages of 100, 150 and 200 mg/kg of the aqueous extract of walnut. At the end of the 13th week, direct blood samples from the heart were taken and the serums were transferred to the laboratory for insulin and glucose level measurements. In order to study the statistical results, one-way

ANOVA technique has been hired. Although a significant reduction in blood glucose levels was observed within the treated group, the increase in serum insulin levels and body weight was so high compared to those of the diabetic group. It was concluded from this study that incorporation of aqueous extract of walnut in diabetic rats considerably decreases their blood glucose levels while it causes a considerable increase in insulin serum levels within the treated group. Not only does aqueous extract of walnut play an important role in prevention and treatment of oxidative stress, but also it can effectively deal with diabetic complications and increase insulin levels.

Keywords: Diabetes, aqueous extract of walnut, insulin, oxidative stress

INTRODUCTION

Diabetes is specified by carbohydrate, lipid and protein disorders. The main biochemical indicator of this decease is chronic hyperglycemia due to partial or complete insulin deficiency and the majority of diabetic patients suffer from cardiovascular disorders, kidney failure, blindness, amputation due to neuropathy and behavioral disorders. Generally, diabetes causes ultimate lipid, protein, and DNA oxidation by making free radicals and oxidative stress; and mitochondrial DNA disorder is one of the most manifest metabolic damages of this disease.[1] Therefore, in order to address such oxidative damages, researchers have incorporated lots of medicines with antioxidant peculiarity such as vitamin A, vitamin C and beta-carotene.[2] Thus, we decided to study the medical effects of aqueous extract of walnut on Streptozotocin-induced diabetes. So far, lots of research has been conducted on leaf or kernel of walnut; yet, no research has been done regarding the walnut fruit itself.

Walnut, scientifically known as" Juglans", is a member of Juglandaceae family. The scientific name of this kind was taken from the Latin word Jovis glans that means Jupiter hazelnut. The most famous walnuts in the world are Iranian walnuts. The Iranian walnut is the only group of this family that is edible and brings financial benefits to their economy. [3]

Walnuts are rich in vitamin E and antioxidants that reduce the devastating effects of diabetes. Also, it possesses anti-cancer effects, regulates blood sugar and lowers blood pressure. Research has shown that increased

insulin levels and oxidative stress results in decreased insulin secretion from the pancreas, decreased number of β cells and also decreased proportion of the islet of Langerhans space to pancreas weight. Destruction of β cells in STZ-induced diabetic rats was totally obvious. Aqueous and alcoholic extracts of walnut leaves and walnut kernels clearly showed the sensitivity to insulin, the secretion of insulin and rebuilding of β cells as well as antioxidant activity in tested rats. In addition, aqueous and alcoholic extracts of walnut leaves and walnut kernels and walnut fruit make the pancreatic superoxide dismutase activities normal. Therefore, an adequate combination of antioxidants can partially destroy the defective β cell function or in other words protect them [4] The hepatoprotective properties of aqueous and alcoholic extracts of walnut leaves and walnut kernels and walnut fruit, as a potential antioxidant, could easily indicate its anti-diabetic properties [5]

Consequently, on the basis of the abovementioned properties, we decided to study the glucose lowering effects of walnut fruit on blood glucose in diabetic rats.

METHODOLOGY

A number of adult male Wistar rats weighing 200-220 grams were collected from the Animal House Department of the Pasteur Institute of Iran, Amol, Mazandaran Province. The rats at the beginning of the experiment were 6 months old. All animals were kept under controlled conditions of light (12 hours of light and 12 hours of darkness) with the ambient temperature of 20-22 °C and relative humidity of 40-60 percent in the Animals' Room of Shahroud University of Medical Sciences to adapt to the new environment. These conditions were maintained during the experiment. During this period, the rats fed on compact food and urban drinking water within specific containers designed for this purpose and therefore, animals had access to sufficient food and water.

The cages were disinfectant four times a week and wood chips of the cages were replaced every day for maintenance purposes [6]

30 adult male Westar rats were classified into five six-member categories: the control group subjected to simultaneous injection of STZ and citrate buffer, the first experimental group including 6 rats that became diabetic through the injection of 55mg/kg of STZ, the second experimental group including 6 diabetic rats treated by aqueous extract of walnut fruit using gavage feeding at doses of 100 mg/kg, the third experimental group including 6 diabetic rats treated by aqueous extract of walnut fruit using gavage feeding at doses of 150 mg/kg and finally the forth experimental

group including 6 diabetic rats treated by aqueous extract of walnut fruit using gavage feeding at doses of 200 mg/kg [7].

Preparation Method of the Liquid Extract of Walnut

Sufficient walnut fruit for extracting was prepared and washed with distilled water and were put in the oven at a temperature of 37 °C to be dried completely. Then, the fruits were completely pulverized in a Chinese pestle and then were put on a magnetic mixer for 24 hours to make a pure solution. The resulting solution was passed through a filter paper and then dried under the right conditions (in the oven at a temperature of 37 °C). Then the dry powder of extract was daily gavaged in needed volume [7].

Receiving the Required Dose of Drug

The rats received medication in daily doses of 100, 150 and 200 mg/kg of aqueous extract of walnut fruit using the gavage feeding method.

Taking Blood Samples from the Animals

At the end of the 13^{th} week, blood samples were taken from the animals to measure the insulin hormone levels in their bodies. At first, the animals were anesthetized through the intraperitoneal injection of Ketamine and Xylazine. Then, the samples were transferred to the laboratory for insulin level measurements. The samples were centrifuged at 4000 rpm for 10 minutes to separate the serum. The serums were transferred to numbered micro tubes by the sampler and were kept in the freezer at a temperature of -20 °C [7].

The Analysis Method of Insulin Hormone and Blood Glucose Measurement

After separating the serum from the blood content through a biochemical kit of insulin (ELISA kit) and after separating the serum from the blood contents using the Pars Azmoon kit, the glucose level of the blood was measured using a spectrophotometer with a wavelength of 546 nm.

Data Analysis

Data analysis was conducted by SPSS 16 Software. The results obtained from the hormone, glucose and body weight changes between the experimental and control categories were expressed as mean ± SD. Statistical analyses to check for significant differences between categories using one-way ANOVA followed by Tukey post hoc test were conducted. The statistical inference border of (05 / 0≥ P) and (001 / p≥ 0/5) and (p≥ 0/1) was considered. Finally, the relevant histograms were plotted incorporating Excel 2013 software.

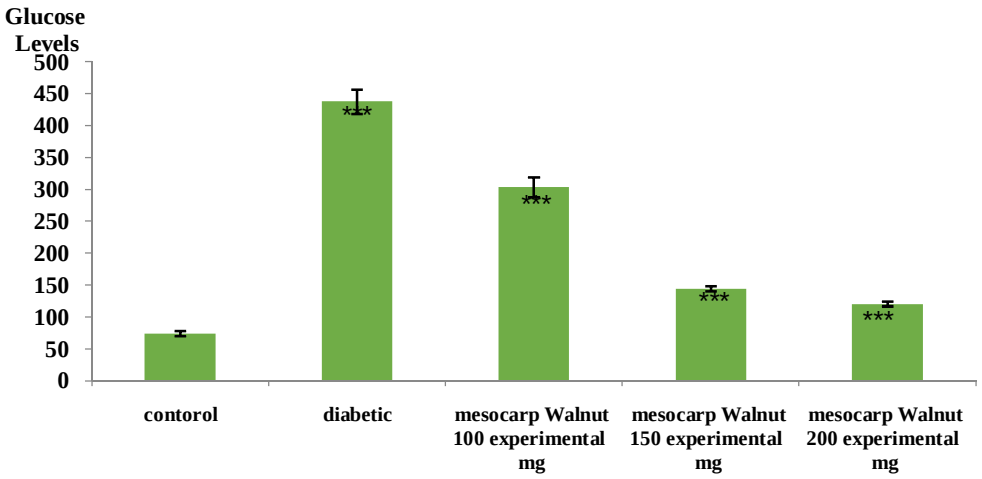

Figure 1: comparison between the mean ± SD of blood glucose levels among the control, diabetic and treated groups with 100, 150 and 200mg/kg of walnut fruit

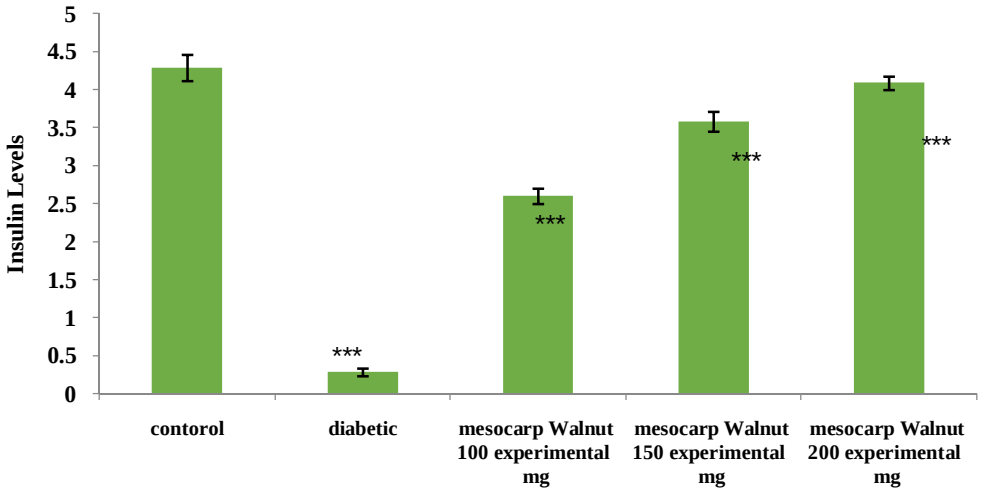

Figure 2: A comparison between the mean ± SD of insulin hormone levels among the control, diabetic and treated groups with 100, 150 and 200mg/kg of walnut fruit

RESULTS AND DISCUSSION

The results of this study have been summarized in Figures 1 and 2 respectively. In Figure 1, the results of the blood glucose mean ± SD among control group, diabetic group and treated group with 100, 150 and 200 ml dose of walnut fruit were compared. In Figure 2, the results of the insulin hormone mean ± SD between control group, diabetic group and treated group with 100, 150 and 200 ml dose of walnut fruit are compared.

According to Figure 1, a significant difference in blood glucose level is observed in diabetic group compared to the control group and also, a significant decrease is observed in the three

diabetic-treated groups compared to that of the diabetic one.

According to Figure 2, a significant difference in insulin hormone level is observed in diabetic group compared to the control group and also, a significant decrease is observed in the three diabetic-treated groups compared to that of the diabetic one.

In diabetic patients, a series of chronic and delayed effects like neuropathy, angiopathy and immunosuppression are caused due to hyperglycemia. From all these complications, increased glucose levels and reduced insulin serum are more common in type 1 diabetic patients. Recent study indicates that walnuts play an effective role in regulating antioxidant properties resulting from streptozotocin-induced diabetes (STZ diabetes). This material and its metabolites act as electron givers due to their electron-rich system and they have redox properties and their anti-oxidant defense is applied on the surface of cell membrane, mitochondria and nuclei within the laboratory environment (in-vitro) and within the body environment (in- vivo)) [8]. Since the anti-oxidant effects have been investigated in previous studies and the effect of walnut fruit on decreasing the diabetic blood sugar and increasing the insulin rate have been addressed in this study, it was concluded that the walnut fruit caused reduced glucose levels, increased sensitivity to insulin and increased body weight within the treated groups (Fig 1-3)

In addition, it has been specified that walnut mesocarp has therapeutic effects on animals with both types of diabetes. In high-fat diet rats, walnut leaf reduces body weight, insulin etc. In type 2 diabetic rats with a high-fat diet and lower dose of STZ, walnut leaf decreases FBG (insulin tolerance). Also, the walnut fruit recover the pancreas deficits of alloxan induced diabetic rats. The walnut fruit inhibits oxygen consumption in cells and by increasing the lactate production, it increases the glycolysis. Moreover, activation of AMPK could result from the mitochondrial inhibition caused by the walnut fruit that could eventually improve the insulin sensitivity [9].

The walnut fruit has shown certain behaviors against fat and carbohydrate metabolism and has lots of effects on glucose homeostasis. In fact, the walnut fruit increases the mRNA expression of insulin receptor through the kinase protein related to cyclin as a promoter in human liver cells and skeletal muscle cultivation [10]. Walnut fruit has shown the ability to protect pancreas and β cells against oxidative stresses in diabetic rats. In many Chinese papers, the effect of walnut fruit on reduction of glucose levels in diabetic patients

with medicines like metformin has been addressed [11]. HbAlc in diabetic patients decreased by 0.2 percent by alcoholic treatment extract of walnut which is comparable with metformin. In fully controlled diabetic patients, alcoholic treatment extract of walnut reduced the HbAlc by 0.8 percent. In addition to hypoglycemia effect, the extract of walnut leaf has positive effects on fat metabolism [12&13]

Walnut fruit controls ATP Synthesis in mitochondria. It has been proven that the walnut fruit directly controls monoamine oxidase in mitochondria. In the current study, mitochondrial control in living cells has been proven [14]. It has been observed that walnut fruit controls the oxygen consumption and increases glycolysis through increasing lactate products as mentioned above. The ATP biosynthesis efficiency due to glycolysis is much lower than its biosynthesis in mitochondria that explains the increased proportion of AMP/ATP. According to these observations, it seems that AMPK activation may be the result of mitochondrial control of walnut fruit. These studies also suggest that this slight control of mitochondrial act might improve the treatment of insulin sensitivity. In another study, inhibition of mitochondrial oxidative phosphorylation during the gene loss process in rats created immunity against insulin resistance caused by diet, diabetes and obesity. This mechanism might be used in fat and glucose metabolism adjustment by walnut fruit.

The walnut fruit may cause decrease of glucose absorption in intestine. It is also amazing that the walnut fruit functions as a glucosidase inhibitor. Glucosidase is an intestine enzyme used for digesting carbohydrates such as sugar and starch into monosaccharide. Inhibition of this enzyme stops the carbohydrate absorption of the diet. Glucosidase inhibitor is one possible alternative for reducing the glucose levels in type 2 diabetes that leads to reduced glucose absorption. Glycosidase activity in Caco-2 cells is inhibited by walnut fruit. Glucose transport from intestinal epithelium also decreases after using the walnut fruit. These two events may cause blood glucose control by walnut fruit that is in agreement with our results (Fig 1)[12]. It is possible that the walnut fruit shows control effects on lipooxygenase and Xanthine oxidase, two important sources of ROS, which is an indication of its anti-oxidase properties. Fakhar *et al* compared the influence of aqueous extract of walnut leaf, wild garlic and tribulus terrestris with benclamide on the glucose levels of diabetic rats [14&13]. They concluded that despite the higher efficiency of

walnut leaf on glucose levels compared to garlic and tribulus terrestris, they could only be used as supplements for typical anti-diabetic medicines and our results on reducing the blood glucose of diabetic rats treated by aqueous extract of walnut leaf are in agrrement with the above mentioned findings [14]

Asgari et al studied the hydroelectric influence of dry walnut leaf in prevention of type 1 diabetes in male adult Wistar rats and in order to measure the insulin levels, glycosylated hemoglobin, LDL, HDL, VLDL and serums were separated and delivered to the laboratory and it was concluded that VLDL and LDL in experimented groups had a significant increase compared to the controlled group and also the serum levels in diabetic rats treated by walnut leaf had a significant increase compared to the control group. Moreover, blood glucose was also reduced compared to the control group which is in agreement with our findings (Fig 1 and 2)

CONCLUSION

It is concluded from this study that the protective effects of the walnut fruit could be summarized as free radicals collector, antioxidant collector and anti-apoptotic figure or blood sugar lowering medicine in diabetic patients. It is anticipated that in the near future, the walnut fruit would function as an oxidant balance developer and antioxidant in treatment of diabetes.

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