Original Article

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Effects of Walnut Leaf Hydro-Alcoholic Extract by Forced Swimming Stress on Serum Levels of Glucose, Insulin and Liver Parameters in Adult Male Rats' Diabetic

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ABSTRACT

BACKGROUND AND OBJECTIVE: Diabetes is a metabolic disorder which is associated with secretion reduction or insulin resistance. Since different stress causes play a crucial role in incidence and chemical drugs side-effects of this disease, this study is aimed to investigate the effects of walnut leaves on insulin, glucose, albumin, AST ALT and ALP enzymes in diabetic and diabetic by forced swimming stress.

METHODS: In this experimental study, 50 adult male rats divided into 5 groups: control, diabetic, diabetic treated with walnut leaves (200mg/kg), diabetic with swimming stress in water 15°C and diabetic treated with swimming stress and walnut leaves. At the end of 21 days injection and phlebotomizing, serum levels of insulin, glucose, albumin and ALT, AST and ALP enzymes have been measured and the related data by using SPSS-18 software, ANOVA and Tukey has been analyzed.

FINDINGS: The results showed that diabetic and diabetic with swimming caused a significant reduction of insulin (4.46±.26,5.06±.14)($p<0.01$) and albumin (1.68±.23,1.68±.23)($p<0.05$).Also, a significant increase in serum glucose, ALP, ALT and AST (467.00±79.53 and 383.80±28.03, 552.20±5.57 and 481.20±4.42, 84.00±8.21 and 88.50±3.83, 84.00±8.21 and 212.20±15.57, 194.60±12.50) ($p<0.001$) compared to the control group has been observed, while walnut leaf extract caused a significant increase in insulin(8.04±0.63)($p<0.01$), albumin(2.22±0.02) ($p<0.05$) and a significant reduction of the serum levels of glucose, ALP, ALT and AST (201.80±17.15, 401.20±22.25, 59.40±2.48 and 130.60±8.88) ($p<0.001$) in diabetic and under swimming stress.

CONCLUSION: The results showed swimming and diabetic causes insulin to be reduced. As a result, glucose and ALP, ALT and AST enzymes have been increased and blood albumin has been decreased. Also, it is observed that walnut leaf extract causes the above mentioned indices to be modified.

KEY WORDS: *Walnut leaf , ALP, ALT , AST, glucose , insulin, albumin , Swimming stress, Rat*.

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Introduction

Diabetes is one of the most common metabolic disorders in the world that the patient is either unable to produce or is resistant to insulin (1). In diabetes, due to increased levels of free radicals and reduced antioxidant capacity of the body and thus oxidative stress there are some problems in the metabolism of proteins, fats and sugars (2). The walnut with the scientific name of Juglans regia is one of the most widely used herbs in traditional medicine. Its leaves are used to treat rheumatic fever, fever, diabetes, and anemia (3). Walnut leaves contain compounds such as glycolic acid, alcohols, potassium, magnesium, barium, carotene and fat and have microbial properties (4).

The green and fresh parts of the walnut plant, especially the leaves, contain antioxidant compounds such as phenolic acids, flavonoids and chlorogenic acid (5). Walnuts increase the antioxidant function of the body and prevent the progression of cardiovascular disease and cancer (7, 6). There are many flavonoids in the walnut leaf such as Quercetin compounds, camphorol, which have a potent antioxidant effect that greatly reduces the intracellular activity of reactive oxygen species (3). Studies have shown that the hydroalcoholic extract of Walnut Flower with antioxidants such as flavonoid compounds increases insulin hormone levels and decreases the amount of glucose and ALT, ALP and AST enzymes in the blood of STZ induced diabetic rats (9, 8).

Studies have shown that regular swimming exercises and the use of arbotin supplementation through the positive regulation of antioxidants and the reduction of lipid peroxidation prevent renal damage induced by oxidative stress in diabetic rats (10). Compulsory swimming in cold water is effective on fasting blood glucose and glucose tolerance testing and increases insulin resistance and weight loss in male rats (11). On the other hand, the results of a study showed that swimming in 35 ° C water reduced blood glucose levels in rats (12). Also, forced stress in the cold water of 15 degrees, increases the serum levels of corticosteroid hormones and glucose in the blood (13). It has been shown that swimming exercises increase the activity of antioxidant enzymes and reduce the level of free radicals in the blood (14).

Given the fact that empirical evidence suggests that stress causes a lot of changes in blood glucose levels (11), and given millions of people around the world suffering from diabetes and possibly one of the reasons for a widespread outbreak of this disease is a type of stress and due to the side effects of chemical drugs used to treat diabetes, this study was conducted to investigate the effect of hydroalcoholic extract of walnut leaves in adult diabetic rats treated with stress of mandatory swimming.

Methods

This experimental study was conducted in 2016 in Shiraz Islamic Azad University with ethics code 13951003-Miau on 50 male Wistar rats weighing between 220 and 210 g and 110 to 100 days old and were prepared from the Center for Animal Breeding and Maintenance Shiraz University of Medical Sciences. During the experiment, all animals were provided with water and packaged foods produced by the feed company of Pars Company in Tehran without any restrictions and were kept in a special room at 22 ± 2 ºC, 12 hours of light and 12 hours of darkness in a special room. For diabetics, intraperitoneal injection of streptozotocin (60 mg / kg) was used (9).

In this study, blood glucose levels, AST, ALT, ALT, and AST enzymes were determined to be more reliable than diabetic rats. After the injection of streptozotocin, blood glucose was measured by blood sampling from the venous region of the demi-dile and measuring the amount of sugar, only animals with a fasting blood glucose greater than 250 mg/dl were considered diabetic to be included in the study (15, 9, 8).

After confirmation of diabetic condition, the animals were divided into 5 groups of 10, including control (non-treated), diabetic control and 3 experimental groups. After administration of streptozotocin and evaluation of diabetic condition, they were treated by gavage with hydro alcoholic extract of walnut leaf 200 mg / kg (16), were subjected to 5 minutes of forced water swimming at a temperature of 15°C (10) and treated by gavage with a hydro alcoholic extract of walnut leaf at a dose of 200 mg / kg along with 5 minutes of forced swimming. For preparing walnut leaf extract, percolation method was used and sufficient amount of walnut leaf from surrounding areas of Shiraz was collected and after identification and confirmation by the botanical department of Shiraz University and after drying was powdered using the electric mill device.

Then the powder sufficiently dissolved out in 200 ml of 96% ethanol and the resulting mixture was kept at room temperature (25°C) for 24 hours to completely soak and then, with the help of an electric device were well stirred to become uniform, and the resulting

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mixture was smoothed by means of a filter and using a rotary mixer, the resulting mixture was concentrated and with a desiccator device all moisture of mixture were taken and an extract with high viscosity was obtained. All prescription for 21 days and was conducted between the hours of 9 to 10 am. At the end of the period, rats were anesthetized by ether and blood sample was taken from their hearts with a syringe 5 ml to measure the serum level of insulin and glucose and AST, ALT and ALP enzymes. To measure the concentration of insulin hormone, the immunosorbent assay method and the insulin test kit manufactured by Diasource Italy (0036 series) and gamma counter can be used. Blood glucose was measured using a glucometer device.

Also, the activity of AST, ALT and ALP enzymes was measured by using the Pars test kit and by photometric method and albumin was measured using serum albumin kit and photometric method. The obtained results from serum levels of insulin, glucose, albumin and ALT, ALP and AST enzymes were analyzed using SPSS 18 software and analyzed by ANOVA and Duncan's test and $p < 0.05$ was considered significant.

Results

The results showed that there was a significant increase in blood glucose levels in animals treated with streptozotocin (diabetic) and diabetic with forced swimming stress ($p < 0.001$). However, in the mean of blood glucose in diabetic animals treated with walnut leaf extract, there was a significant decrease in diabetic and diabetic groups with forced swimming stress ($p<0.001$) (Table 1). In addition, the results of this study showed that in diabetic group and diabetic group treated with forced swimming stress, there was a significant decrease in the level of insulin compared to the control group and in diabetic group and diabetic groups treated with forced swimming stress, the activity of ALP, ALT and AST enzymes increased significantly at the level of $p < 0.001$ and the serum level of albumin decreased significantly at the level of $p < 0.05$ compared to the control group (Table 1).

Table 1. Comparison of the effect of walnut leaves extract and forced swimming stress alone and in combination with serum sugar, insulin, albumin, AST, ALT and ALP enzymes.

Groups	Glucose (g/d) (mean±SD)	Insulin (ng/ml) (mean±SD)	AST (IU/L) (mean±SD)	ALP(IU/L) (mean±SD)	ALT(IU/L) (mean±SD)	Albumin (g/dl) (mean±SD)
control	87.00±3.20	9.52±1.56	120.00±8.03	351.20±4.20	51.00±3.11	3.14±0.58
Control(diabetic)	38.80±28.03***	5.06±0.41**	194.60±12.50***	552.20±5.57***	84.00±8.21***	1.68±0.22*
Experimental 1 (Diabetic+Walnut Extract 200 mg/kg)	201.80±17.15*$	8.04±0.63&	130.60±8.88#	401.20±22.25#*	59.40±2.48#	2.22±0.02
Experimental 2 (diabetic+forced swimming stress)	46.00±79.53***	4.46±0.26**	212.20±15.57***	481.20±4.42***	88.00±3.83***	1.68±0.23*
Experimental 3 (diabetic+walnut extract, 200 mg / kg and forced swimming stress)	355.60±29.52***	5.96±0.77**	141.00±11.16#	421.20±23.02#	61.00±3.78#	2.20±0.02

*** ($p<0.05$), **$p<0.01$ and *$p<0.001$ showed significant difference with control group, $p < 0.001$=#, significant difference with control and experimental groups 2, $ = $P < 0.001$, significant difference with other groups, $p < 0.001$=& significant difference between the control and experimental groups

Discussion

The results of this study showed that administration of streptozotocin can increase blood glucose levels, ALT and AST enzymes and decrease serum levels of insulin and albumin. However, in diabetic rats, forced swimming stress had no significant effect on serum levels of insulin, blood glucose, albumin, ALT and AST. But administration of the walnut leaf extract in diabetic rats increases the serum level of insulin, albumin, and decreases the blood glucose, ALT and AST. Together with the results of this study, another study also showed that 60-day consumption of Walnut leaf extract would improve blood glucose levels in patients with type 2 diabetes (10). Another study also found that 28-day administration of Walnut leaf extract

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reduced levels of glucose and glycosylated hemoglobin (17). Investigations have shown that diabetes mellitus increases the level of malondialdehyde (MDA) and decreases the level of total antioxidant capacity (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) enzymes, while the alcoholic extract of the Walnut leaf reduces the serum levels of malondialdehyde and increase the activity of SOD, CAT, and GPX enzymes (18). Therefore, in the present study, oral administration of alcoholic extract of walnut leaves in streptozotocin-diabetic rats has been shown to increase its anti-diabetic effects by increasing the antioxidant capacity of the body. Diabetes mellitus due to streptozotocin can cause functional and structural changes in various cells of the body, including the liver (19).

The possible increase in ALP, ALT and AST enzymes is due to functional and structural changes in the liver in diabetic groups. El-Demerdash et al. showed that increased plasma levels of lactate dehydrogenase, alkaline phosphatase, acid phosphatase, AST, and ALT are likely to occur due to liver function impairment (20). Masjedi et al. also found that in diabetic patients, necrosis can be observed in the liver leading to increased serum levels of transaminases, such as AST, ALT, and ALP (21). Therefore, in the present study, diabetes through liver failure or tissue necrosis likely leads to leakage of AST, ALT, and ALP from liver cytosols into the bloodstream resulting in increased levels of these enzymes in the blood. Increasing protein catabolism along with gluconeogenesis and the formation of urea in diabetes may be responsible for increasing these transaminases in the blood (23, 22). Since insulin suppresses the genes that produce gluconeogenic enzymes, and given that ALT is also a gluconeogenic enzyme, in the present study, the increase in ALT levels in diabetic rats is probably due to decreased insulin levels as a result of prescribing streptozotocin, which cannot be associated with liver damage and the reduction of this enzyme in animals treated with walnut leaf extract can be due to an increase in insulin hormone (24).

In the present study, there was a significant increase in the activity of AST, ALT, and ALP in the diabetic rats compared to other groups, and treatment with Walnut leaf extract significantly reduced their activity. Therefore, the results of this study indicate that treatment of diabetic rats with hydroalcoholic extract of Walnut leaves could reduce liver enzymes. Walnut leaves with zeoglon flavonoids, quercetin galactoside, quercetin pentoside derivatives, quercetin arabinoside, quercetin xyloside, quercetinraminozide and pentoside derivatives has high antioxidant properties that by increasing the antioxidant capacity of the body by collecting free radicals preventing from the destruction of various cells in the body and has a protective effect on the body against damage from toxins and free radicals (25). Therefore, in the present study, walnut leaf extract by increasing the antioxidant capacity and collecting free radicals prevented from their destructive effects on the liver tissue and therefore resulted in reduction in the amount of AST, ALT and ALP enzymes. In this study similar to results of another study indicated that albumin concentration decreased significantly in diabetic groups and diabetic groups treated with forced swimming (26).

A study found that various diseases, especially liver disease and damage to liver cells, reduce the synthesis of albumin and total protein (27). Therefore, in the present study, decrease in albumin concentration in diabetic groups can be attributed to damage to liver cells. But in groups treated with hydroalcoholic extract of walnut leaves, it is somewhat prevented from reducing it, probably due to the properties of the liver protection of the walnut leaf extract. It was somewhat consistent with the results of this study in another study that alcoholic extract of walnut leaves protecting the liver against oxidative damage caused by carbon tetrachloride by having antioxidant activity and sweeping free radicals (16).

In other words, it can be said that this effect is probably due to the presence of flavonoids, especially quercetin compounds. The results of this study showed that diabetes alone or with forced swimming stress in cold water increases serum levels of glucose, AST, ALT and ALP enzymes, as well as decreases insulin and albumin protein levels. But Walnut leaf extract probably prevents the effects of swimming stress and diabetes on above mentioned factors due to its highly antioxidant compounds.

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