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**Original Article**

# Investigation into the hypoglycemic effect of hydroalcoholic extract of Ziziphus Jujuba Leaves on blood glucose and lipids in Alloxan-Induced diabetes in rats

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## Abstract

**Background**: Diabetes mellitus is the most prevalent endocrine disease that increases blood glucose and lipids. This disease affects cardiovascular system, kidneys, nervous system and eyes. Studies have indicated some herbal extracts have anti-diabetic effects, and can be used in diabetic patients for reducing blood glucose. Hence, in the present study, we decided to induce diabetes, which was similar to human type1 diabetes, in experimental rats and, after diabetes verification, we evaluated hypoglycemic effect of hydro-alcoholic extract of Ziziphus Jujuba leaves and blood lipoproteins (LDL, HDL, and VLDL), triglyceride and total cholesterol changes.

**Methods**: In the present study, 30 adult male rats were assigned to 3 groups as follows:
Group 1 was treated by saline (2 ml /kg, i.p.)
Group 2 was treated by alloxan monohydrate (120 mg/kg, i.p) for 3 days alternately. Then, blood glucose was evaluated, and diabetes verified.
Group 3 was treated by alloxan monohydrate for 3 days alternately and, after blood glucose evaluation and diabetes verification, animals received hydro-alcoholic extract of Z. Jujuba (100mg /kg, i.p.) for 5 days alternately.
After 48 hours, the animals were anesthetized and the blood was collected into a tube, then, levels of serum glucose, lipoproteins (HDL, LDL, and VLDL), triglycerides, and total cholesterol were evaluated by enzymatic kits.

**Results**: The analysis variance results indicated significant reduction ($P = 0.001$) of glucose– triglyceride– cholesterol and VLDL levels in group 3 in comparison with group 2. Z. Jujuba also increased HDL levels significantly ($P=0.001$) in comparison with group 2. Also, the extract reduced diabetic rats LDL level, but it wasn't significant ($P=0.12$) in comparison with group 2.

**Conclusion**: According to the results obtained, it was concluded that, Z. Jujuba leaves can be used in diabetics for the purpose of glucose and lipid reduction.

**Key words**: Ziziphus Jujuba, lipid, glucose, diabetes

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## Introduction

Diabetes mellitus is the most prevalent endocrine disease which leads to blood glucose increase (hyperglycemia) and disturbance of carbohydrate, lipid and protein metabolism. This disease is created as a result of disturbance of insulin secretion or action or both, which in turn, affects total organs of the body. Considering side effects of blood glucose-reducing drugs and their expenses, finding compositions with low side effects that can, at the same time, reduce blood glucose seems necessary. Attention to diabetes mellitus is necessary because number of diabetic patients is increasing and diabetes has various side effects [1,2]

Use of medicinal plants in medicine is increasing because of their widespread use and for their curing various diseases. Recent studies have indicated that some herbal extracts have anti-diabetic effects, thus can be used in diabetic patients in order to reduce blood glucose, for example hypoglycemic effect of Dandelion alcoholic extract have been recorded by some researchers [3]. Aqueous extract of Vernonia Clorota and Avocado alcoholic extract demonstrated hypoglycemic potential [4,5]. Then, the purpose of the present study is finding a composition that could reduce diabetic patients' blood sugar with the least side effects.

## Methods

### 1. Animals

We used mature male rats (Rattus Norvegicus Allivias). The animals were bought from animal room of Isfahan University of Medical Sciences, and were kept in the Isfahan Payame Noor University animal room. After the adaptation period was over, each group of rats was weighted and marked, and then treated by the specified dose of materials.

For inducing diabetes in rats, we used alloxan monohydrate (made in Merck factory of Germany) 120 mg/kg (i.p.) solved in saline (9 % sodium chloride solution).

Alloxan injection causes apoptosis and necrosis in Langerhans beta cells of pancreas, that has been used for diabetes induction in different kinds of animals [6] and is similar to human type 1 diabetes [6].

In this study, animals were assigned to 3 groups having the following characteristics:

1) Group 1 (normal control): was treated by saline (2 ml/kg, i.p.)

2) Group 2 (diabetic control): was treated by alloxan monohydrate (120mg/kg, i.p.) for 3 days alternately. Then, blood glucose was evaluated, and diabetes was verified. For diabetes verification, we obtained blood by use of hematocrit tube, via a method called Stone Method, such that venous blood was collected from the internal edge of the orbital cavity. For this to proceed, we held rats by hand, then the end of hematocrit tube entered slowly into the internal edge of the eye and, with circular motion, the tube end was directed into the internal sinus of the eye; after a few large blood drops, the hematocrit tube was removed, and the blood was collected in the eye beneath the experimental tube.

In this way, you can get venous blood of orbital sinus more than once, which is suitable for primary experiments (diabetes verification). Then blood glucose was evaluated and recorded by enzymatic kits.

3) Group 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 Z. Jujuba (100 mg/kg, i.p.) for 5 days alternately. And after 48 hours, the animals were anesthetized and the blood was collected in tubes, then, levels of serum glucose, lipoproteins (HDL, LDL, and VLDL), triglyceride, and total cholesterol were evaluated by use of enzymatic kits.

### 2. Z. Jujuba Hydro-Alcoholic Extract Preparing

First Z. Jujuba leaves were bought from Isfahan Department for Natural Resources which after being washed, were powdered by mill, and 100 gr were isolated by a digital balance, then were sloped into a 1-litre beaker and 400 cc ethanol 96% was added to cover the surface of the powder**,** beaker was positioned on the shaker for 24 hours. Then, the solution was filtered, and again ethanol 75% was added to the remained waste, and was positioned on the shaker for 12 hours. Finally, filtered solution concentrated by Rotavapor (which was adjusted on temperature 50 $^{0}$ C and rotated with a speed of 90/min) up to 1/$_{3}$ the primitive volume. For proteins isolation and material refining, the filtered solution, after being **decantated** 3 times by chloroform, was positioned in incubator at 50 $^{0}$ C. After a few days, the powder was ready and included net and effective material of the plant.

### 3. Statistical analysis

In this study, we ran a one-way ANOVA to see if there were any statistically significant differences among the three groups of rats. The data collected were put into SPSS and the relevant figures were drawn with Excel.

## Results

### 1. Glucose Level of Group 3 (the Extract Group) Compared with Other Groups

According to fig.3.1, mean difference between extract group and diabetic control is statistically significant (P= 0.001), thus, Z. Jujuba extract has significantly reduced glucose level. But, mean differences between diabetic control and normal control is significant too (P= 0.001), that indicates that Z. Jujuba could reduce blood glucose from 767.82 mg/dl to 250.94 mg /dl but not to normal control level.

### 2. Triglyceride Level of the Extract Group Compared with Other Groups

Analysis of variance results indicates that mean difference between the extract group and diabetic control is significant (P=0.001) and, according to the fig.3.2, it is clear that Z. Jujuba has reduced triglyceride level significantly. It is interesting that the mean difference between the extract group and the normal control is not significant (P>0.05), which indicates that Z. Jujuba extract has reduced triglyceride level to normal level.

### 3. Cholesterol Level Comparison of Extract Group with Other Groups

Analysis of variance results show cholesterol mean difference between the extract group and the diabetic control is significant (P=0.01), then Z. Jujuba extract reduced cholesterol level to some considerable but the mean difference between the extract group and the normal control, was not statistically significant. As it can be observed in fig.3.3 Z. Jujuba extract has reduced blood cholesterol to lower level of normal control.

### 4. VLDL Level of the Extract Group Compared with Other Groups

Analysis of variance results and fig.3.4 show that the mean difference between the extract group and the diabetic control is significant statistically (P=0.001), which indicates that the extract has reduced plasma VLDL significantly. No statistically significant differences were noted between the extracts and the normal control group.

### 5. LDL Level of the Extract Group Compared with Other Groups

Analysis of variance results show that the mean difference between the extract and control groups was not significant (P=0.12), which indicates that the extract could not reduce LDL level significantly; however, it reduced blood LDL from 39.04 mg/dl in diabetic rats to 29.68 mg/dl. According to the fig.3.5 the difference between the extract and the control groups was not statistically significant (P=0.29).

## 6. HDL Level of the Extract Group Compared with Other Groups

Analysis of variance results show that the mean difference between the extract and diabetic control groups is significant (P=0.02), which indicates that the extracts have increased HDL level in diabetic rats. Mean difference between the extract and normal group is significant too (p =0/04) which indicates that the extract increased HDL level from 26.58 in diabetic rats to 38.5mg/dl, but could not increase it to normal level (fig.3.6).

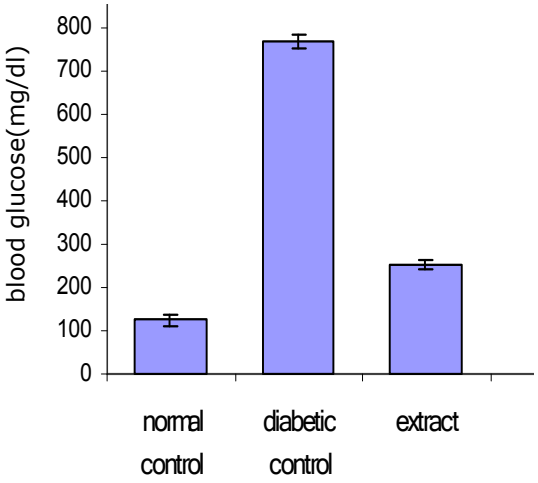


**Fig.3.1- Glucose level of the group 3( extract group) compared with other groups**

Statistical method: ANOVA

Sampling number: each group 10 male mature rat

Group 2,3: P =0.001

Group 1,3: P =0.001

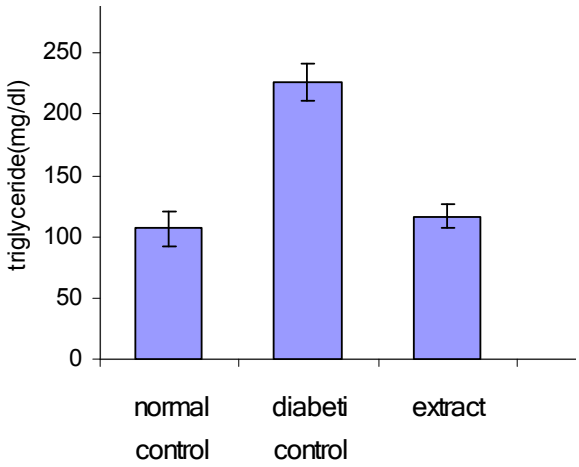


**Fig.3.2- Triglyceride level of the extract group compared with other groups**

Statistical method: ANOVA

Sampling number: each group 10 male mature rat

Group 2,3: P =0.001

Group 1,3: P >0.05

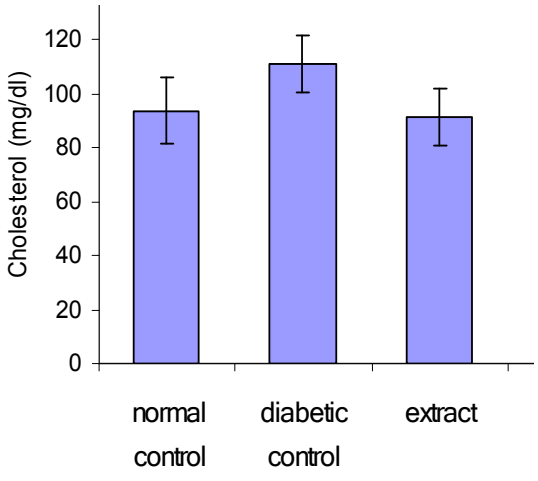


**Fig.3.3- Cholesterol level of the extract group compared with other groups**

Statistical method: ANOVA

Sampling number: each group 10 male mature rat

Group 2,3: P =0.01

Group 1,3: P >0.05

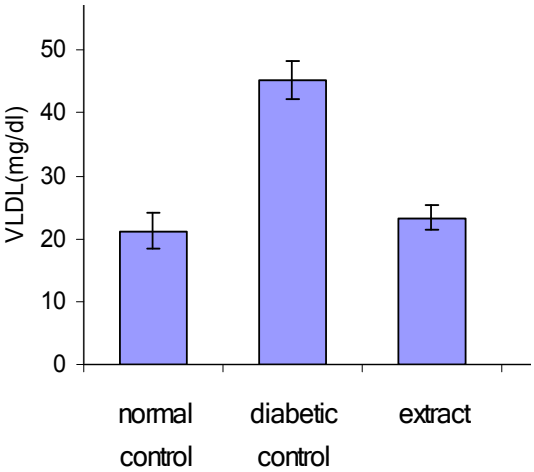


**Fig3.4- VLDL level of the extract group compared with other groups**

Statistical method: ANOVA

Sampling number: each group 10 male mature rat

Group 2,3: P =0.01

Group 1,3: P >0.05

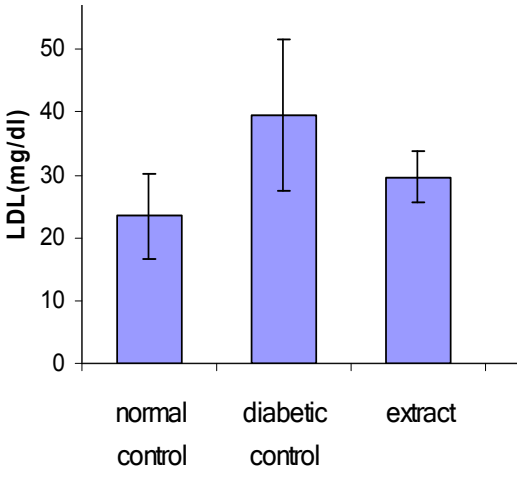


**Fig.3.5- LDL level of the extract group compared with other groups**

Statistical method: ANOVA

Sampling number: each group 10 male mature rat

Group 2,3: P=0.12

Group 1,3: P =0.29

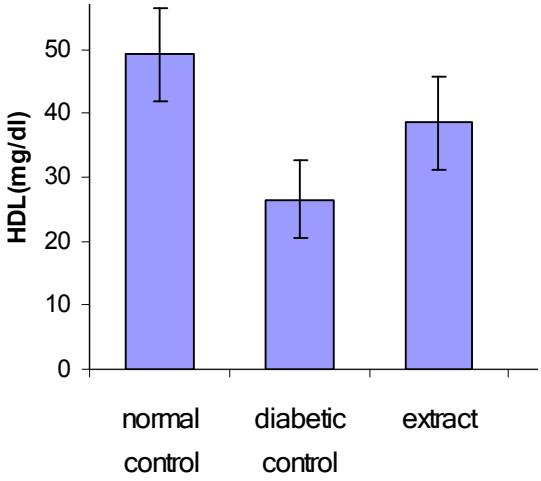


**Fig3.6- HDL level of the extract group compared with other groups**

Statistical method: ANOVA

Sampling number: each group 10 male mature rat

Group 2,3: P =0.02

Group 1,3: P=0.004

## Discussion

In the present study plasma glucose increased by degeneration of Langerhance beta cells, that has been created by alloxan monohydrate, is similar to Mazumdar B, Bath JD, Byung Hyung findings [7], and blood sugar reduction by hydro-alcoholic leaf extract of Z. Jujube is similar to Fahdettin, Kelestimur, Aydin Erenmemisogl findings [8]. One of the extract effects probably is liver phosphorylase inhibition, which inhibits glycogen storage breakdown in hepatic cells and increases the enzyme activity that result in glycogen synthesis improvement.

Following alloxan injection, and blood sugar increasing, triglyceride increased too, that is similar to Zhang XF and Tan BK findings, and demonstrates insulin role in lipids metabolism adjustment [9]. The extract caused triglyceride reduction according to Ignacimutu and Amarlaj study [10]. Probably, the mechanism of triglyceride reduction by the extract is improved glycemic control and blood sugar reduction, glucose consumption instead of lipids for energy production, increase of acetyl CoA (resulted from pyruvic acid), which enters Krebs cycle (instead of entering triglyceride synthesis pathway) and causes final metabolism of glucose [9]. By triglyceride reduction by the extract, VLDL level reduced significantly too, that it is similar to Amarlaj and Ignacimthu findings [10]. About this event one 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.

Plasma cholesterol concentration increase following alloxan injection is similar to the results of investigations of Moorthy, Yadaw, and Baquer [11]. Alloxan injection reduced cholesterol level. This finding corresponds to lgnacimuthu and Amarlaj's study [10, 12].

In diabetic rats, LDL level increased and HDL level decreased which corresponds to Abou-Seif MA, Ishla, Bhart Nagar, Winocour, Durrington, and Yussef AA findings [13, 14]. Hydro-alcoholic extract of Z. Jujuba leaf reduced LDL and increased HDL.

Considering VLDL involves LDL particle generation indirectly, and then plasma VLDL increases and results in plasma LDL increase. 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 Z. Jujuba leaf could reduce triglyceride level, and then by decrease of triglyceride level, increase of HDL level should be expected. With the glucose metabolism improved, protein metabolism is directed into anabolic instead of catabolic process, which, then, results in synthesis of proteins such as Apo-A1 that constitute 70% of HDL structure; this, in its own turn, results in increase of HDL concentration in rats [15, 16].

## Conclusions

According to the results, defines Z. Jujuba leaf can be used as a drug for reducing blood sugar and lipids in diabetic patients. Also considering HDL reduction in diabetics is a risk factor for cardiovascular diseases and each 0.1 mmol/lit reduction in HDL level increases the risk of cardiac diseases 1.5 fold [17], then, this study suggests that Z. Jujuba extract can reduce the risk of cardiovascular diseases.

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