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# Effect of ethanol- extract of pumpkin (*Cucurbita ficifolia*) leaves on blood glucose, lipids and lipoproteins in diabetic rats with alloxan- monohydrate

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**ABSTRACT**

*Diabetes is the most common endocrine disease which blood sugar and fat increases followed. Research has shown that some plant extracts have anti-diabetic and so people with diabetes to lower blood sugar, be used therefore, in this study it was decided to establish an experimental diabetic rats, the same situation occurs with type 1diabetes in rats. And the impact of diabetes, hypo-glycemic effect of hydro alcoholic extract of leaves, pumpkins (Cucurbita ficifolia) and metabolic changes in glucose, triglycerides and total cholesterol lipoproteins tend nous be reviewed. In this study rats were divided into 3 groups of 10 each as follows:1-A control injection of saline (intraperitoneally) 2-Diabetic controls: Alloxan monohydrate injection (intraperitoneally) 120 mg/kg in 3 days, after this period of frequent blood glucose monitoring and diabetes was confirmed).3-Cucurbita ficifolia: injecting Alloxan monohydrate 120 mg/kg (intraperitoneally) at 3 days intermittent. After this period, blood glucose monitoring and diabetes was confirmed after confirmation of diabetes, Injection of ethanol extract of pumpkin leaf 100 mg/kg, intraperitoneally and 5 days was staggering. After 48 hours, animals were anesthetized and blood samples were taken from all groups and levels of glucose, lipoprotein, and total cholesterol and triglycerides were determined by enzymatic kits. The results of ANOVA showed that the leaf extract could decrease serum glucose, triglycerides, and VLDL cholesterol levels in diabetic rats compared with diabetic control group significantly (P=0/001).Cucurbita ficifolia are also significantly HDL (p = 0/12) in the blood of diabetic rats compared with the control group increased. In the extract above, the low level of LDL in diabetic rats compared with controls, but the difference was not significant(p=0/12). Considering the findings that the pumpkin leaves can reduce diabetes Sugar, blood fat used.*

**Keywords:** *Cucurbita ficifolia*, Sugar, Lipid levels, Diabetes, Rats, VLDL, blood glucose, LDL

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## INTRODUCTION

Diabetes is the most Prevalent endocrine diseases that its characteristics are the glycerin Levels increase in blood (Hyperglycemia) and Carbohydrates, Lipid and protein metabolism disturbance. Diabetes mellitus is a metabolic disorder as old as mankind and its incidence (4 to 5%) is considered to be high all over the world (16, 19). This endocrine disorder results from abnormal metabolism of carbohydrates, fats and proteins and causes the increase in blood glucose values. Hepatic and renal failure is the main cause of death in diabetic patients (7, 17). This disease emerges in the effects of Insulin secretion, its operation disturbance or both of them. In the duration of disease involve all of body systems and organs. With notice to side effects and expensiveness of chemical drugs for reduce glycerin levels; access to compounds that reduce the side effects and reduce the levels of glycerin is essential. We must notice this disease because it has various effects and diabetes is increasing in the World (3, 9). As Herbal drugs used for treatment of diseases from the past and used the served herbs and its extractions too and had miraculous patterns some of these herbs, we can see the increasing usage of these herbs for treatments (13). Many studies in the recent years showed that some of herbal Compounds have antidiuretic effects, So We can use them for reduce the

glycerin most of investigators demonstrated that Teraxacum leaves extraction has hypoglycemia effect, specially its blue extraction(8). Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study suggests that alloxan does not cause diabetes in humans (10, 11). Others found a significant difference in alloxan plasma levels in children with and without diabetes Type 1(6). So that the Purpose of this study is access to compound that has hypoglycemia effects with minimum side effects and use it for reduce the glycerin levels diabetes.

## MATERIALS AND METHODS

In this investigation, we used 40 female rats of wistar race that had $200 \pm 20$ g and 12 weeks age. Mice maintained in the animals room, Standard Condition about $21-23^{\circ}c$ environment temperature, 70 percent relative and 12 hours for Lighting, 12 hours for darkness light cycles. We used Standard food for rats nutrition with Ad libitum method (in this method, nourishment is available 24 hourly) and Water was Sufficient for them. After Compatibility period passage for new condition, Rats distributed in several groups and indicated. Injection for each group with indicates doses were done. For investigation, we used Alloxan monohydrate in rats. This Compound used 120 mg/kg rats body weights, in the physiologic serum Solution form (Sodium Chloride 9/1000) and injected in the gall bladders of rats. This injection resulted increase the necrosis and Apoptosis process in the Langerhans islands cells in the pancreas (14, 20). This method has used for creation of diabetes in the most animals. With this injection provide the Similar Conditions of human's diabetes type 1 in the rats.

In this investigation we grouped rats in the three groups and 10 rats in each group:

**1) Witness group:** The injection of physiologic Serum in the form of intra peritoneal.

**2) Control group:** Alloxan monohydrate 120 mg/ kg injection sequentially in the three days (for diabetic proof, we used stone method for bleed by hematocrit tube. In this method we collected the blood form the Orbital Sinus veins in the medial canthus of rats eyes. For this purpose, we fixed the rats with our thumb and forefinger and slowly interred the hematocrit tube in the eye cavity, the capillaries of this section are sensitive and rupture in the pressure and the blood Spout from free end of tubes. After collected of Some Large blood drops, we put the hematocrit tube and used Lab tube for Collect of blood. In this method we can collect the blood of rats Orbital Sinus Veins in the several order that it is useful method for assure the trial process. After that we used enzyme kits for investigation and record of glycerin levels.

**3) *Cucurbita ficifolia* leaf group:** Alloxan monohydrate injection 120 mg /kg in the three days sequential. After this period we used the process of second group and confirmed the diabetes, after that we injected 100 mg/kg from hydro-alcoholic extraction of *Cucurbita ficifolia* leaves intra peritoneal for five days. 48 hours after the last injection blood samples were taken from all of groups and we used of resulted Serum for measure of glucose, total Cholesterol, triglycerides and blood Lipoproteins (HDL, VLDL, LDL) by the enzyme kits.

***Cucurbita ficifolia* leaves hydro-alcoholic extraction preparation method:**
After washing, with use of grind we prepare the *Cucurbita ficifolia* leaves Powder, and with Sensitive pointer separate 100 g and pour it in the Erlenmeyer flask and add 400 cc of 96% alcohol, we put the Erlenmeyer flask in the Shaker for 24 hours, then with funnel and filter paper filtrate the Solution, again pour 75% alcohol, and put the Erlenmeyer flask in the shaker for 12 hours.

The filtrated Solution Concentrated to 1/3 original Volume with a distiller in the Vacuum. For proteins isolation and filtration, the filtrated Solution decant by Chloroform. The decanted Solution put in the incubator on the up $50^{\circ}c$ . After Some days, the dry powder resulted that Contains of filtrate and effective material.

**Statistical analysis**
The statistical package for social sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained

were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. P<0.05 was considered statistically significant.

## RESULTS

**Serum concentration of Glucose Comparison in the Cucurbita leaf with other groups**

As shown in the Fig.(1) , mean deviation in the Cucurbita Leaf group and diabetic Control is Severely meaningful (p=0.001) and we can Conclude that Cucurbita hydro-alcohol extraction Considerably reduce the glucose. But mean deviation in the Cucurbita group and witness group is meaningful too (p=0.001). We conclude that Cucurbita leaf extraction Can reduce Considerably glucose from 767.82 in the diabetic rats to 250.94 mg/dl, but can't meet the witness group levels. Fig.(3)

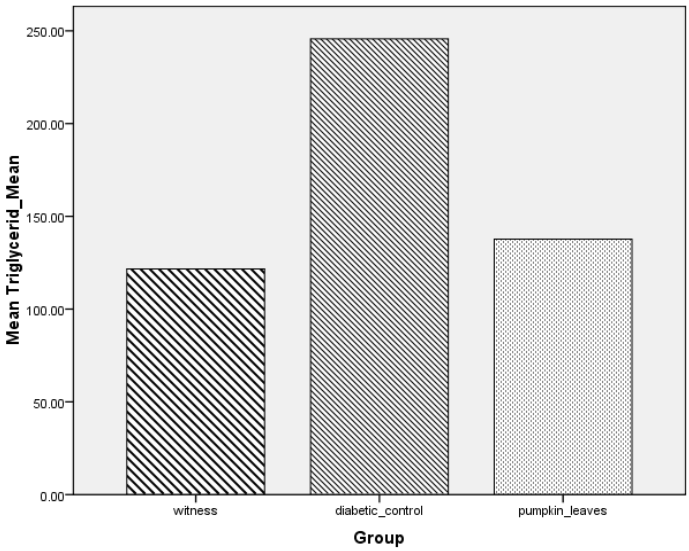


**Figure1.triglyceride concentration levels in the serum comparison in the Cucurbita leaf group with other groups**

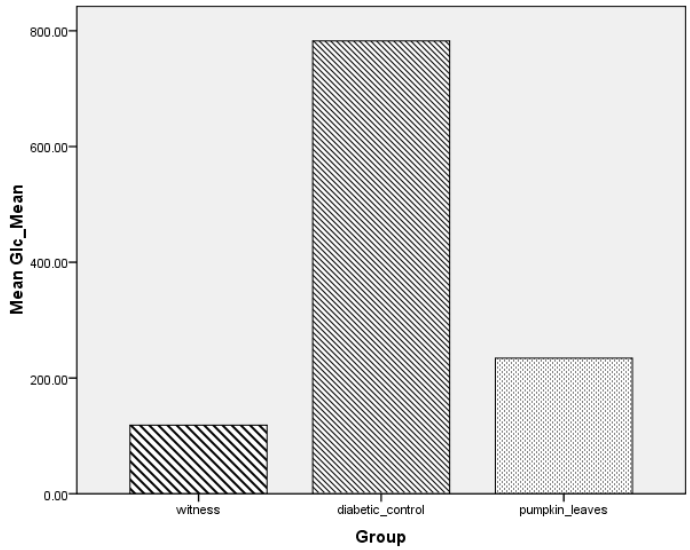


**Figure2. Glucose serum concentration comparison in the Cucurbita leaf group with other groups**

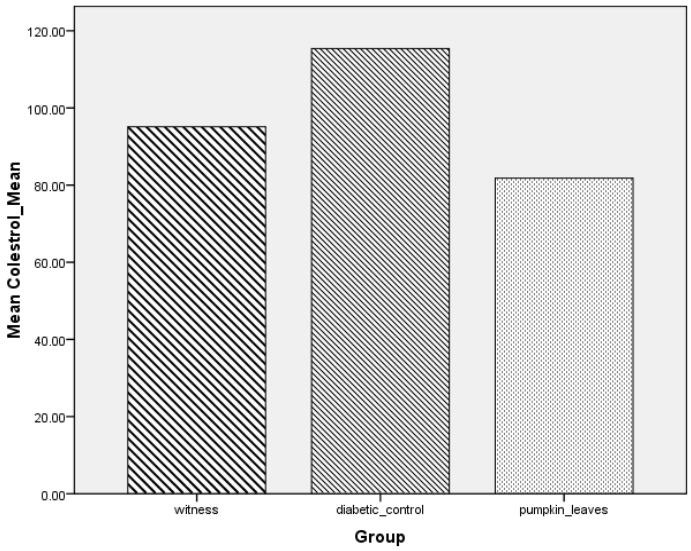


**Figure 3. Concentrates Cholesterol levels in the serum comparison in gourd leaf group with other groups**

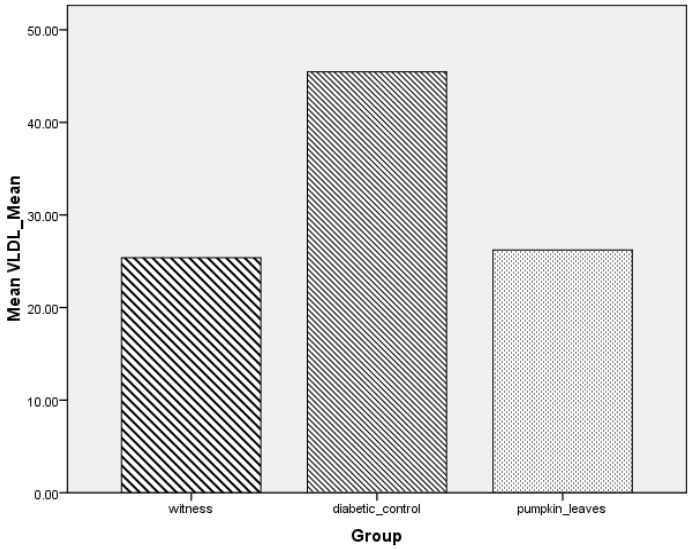


**Figure 4. VLDL serum Concentration levels comparison in the Cucurbita leaf group with other groups**

**Triglyceride Serum concentration comparison in the Cucurbita leaf with other groups**

The result of variance analysis shows that the mean deviation in the Cucurbita leaf group and diabetic control group is severely meaningful (p=0.001) and Fig. (2) shows that the Cucurbita leaf can censurably reduce triglyceride level and it's notable that mean deviation in the censurably leaf group and witness group is meaningful (P>0.05) and this shows that censurably leaf can reduce the triglyceride level as well as witness group Fig.(1) .

**Concentrate Cholesterol levels comparison in the gourd leaf group with other groups**

The results of variance analysis show that the mean deviation of cholesterol in the Cucurbita leaf group and diabetic control is meaningful (P=0.001) and this means that Cucurbita leaf reduce considerably cholesterol levels. But in the Cucurbita leaf group and witness group we can't see the meaningful mean deviation (P>0.05) with notice to Fig. (3) we can conclude that Cucurbita leaf reduce the Cholesterol level down of the witness group Fig.(2).

**VLDL concentration level of blood comparison in the Cucurbita leaf group with other groups**

The results from variance analysis show that the mean deviation in the Cucurbita leaf group and diabetic control group statistically is meaningful (P=0.001). This shows that Cucurbita leaf considerably reduce the VLDL levels in the plasma. There is no meaningful statistical deviation in both groups (P>0.05) and we conclude that Cucurbita leaf can reduce the VLDL levels as well as witness group Fig.(4).

**HDL serum Concentration comparison in the Cucurbita group with other groups**

The results of variance analysis show that the mean deviation in the Cucurbita leaf group and diabetic control group is statistically meaningful (P=0.02) and we conclude that Cucurbita leaf considerably reduce HDL levels in the diabetic rats. Mean deviation in the Cucurbita leaf group and witness group statistically is meaningful (P=0.04). We can conclude that although Cucurbita could increase HDL levels in the rats from 26.58 to 38.50 mg/dl, but it couldn't meet to witness group Fig.(5).

**LDL serum Concentration level comparison in the Cucurbita leaf group with other groups**

The variance analysis results show that the mean deviation in the Cucurbita leaf group and diabetic control group is not significant (P=0.12) and we conclude that Cucurbita leaf couldn't reduce considerably the LDL level in the blood, although it can reduce the LDL level in the diabetic rats from 39.04 mg/dl to 29.68 mg/dl. There is no significant mean deviation in the Cucurbita leaf and witness groups (P=0.29) that Cucurbita leaf could reduce LDL level as well as witness group Fig.(6).

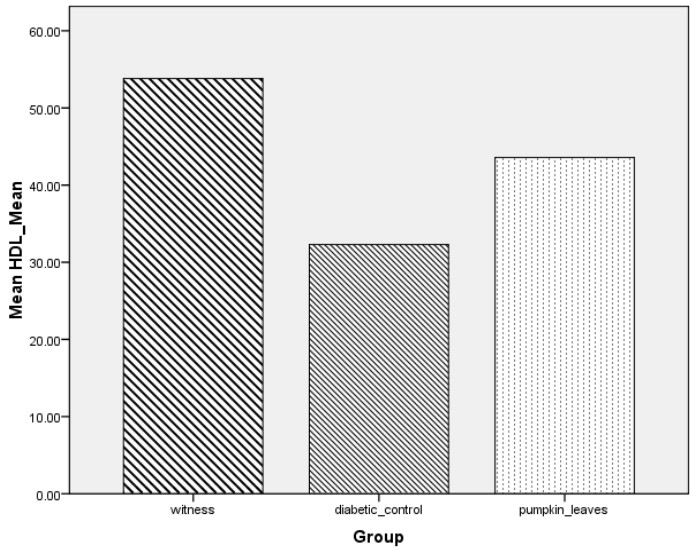


**Figure 5. HDL Concentration levels comparison in the Cucurbita leaf group with other groups**

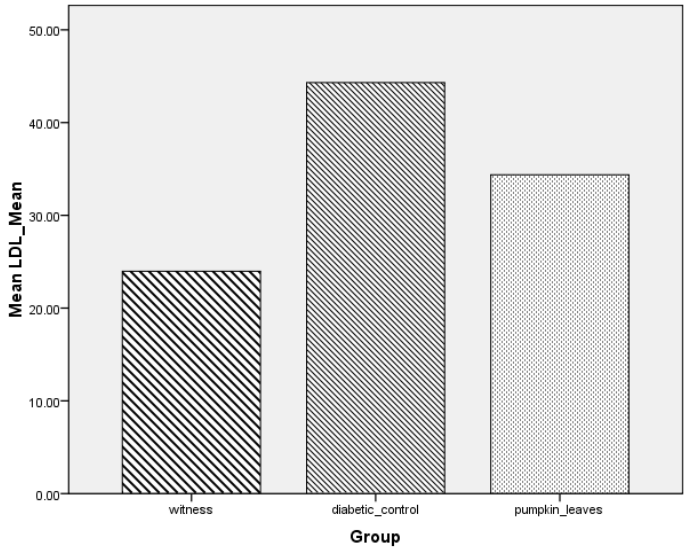


**Figure 6. LDL Concentration levels in the serum comparison in the Cucurbita group with other groups**

## DISCUSSION

The findings of this survey about glucose increase with Langer Hansen β-cells destruction by the Alloxan monohydrate were similar with Byung JD, Hyung(5) findings of glucose reduce by Cucurbita leaf hydro-alcohol extraction were similar to findings of Fahlettin kelestimur, Aydin Erenmemisoglu (4). One of the effects of this extraction is prevent of liver Phosphorylation so it could prevent break up of glycogen in the liver cells and it could increase glycogen synthesis with increase the glucose levels in the diabetic rats after Alloxan injection, triglyceride levels increase too that is similar to Zhang XF, Tan BK findings(21). It shows the Insulin pattern in the Lipids metabolism adjustment. Cucurbita leaf hydro-alcohol extraction, according to Amarlaj can reduce the triglyceride levels. Considerable reduce in the triglyceride level by Cucurbita leaf hydro-alcohol extraction can justify here by: with control the glycerin level and glucose reduction by Ginger extraction, the usage of glucose instead of lipids for energy production is increased and Acetyl Co-enzyme A from valproic acid instead of inters to triglyceride synthesis process, inters to krebs cycle and ultimately starts the glucose metabolism(21).with triglyceride reduce by Cucurbita leaf, according to Amarlaj, VLDL levels significantly reduce.

We must say that intracellular increase of triglyceride resulted the VLDL synthesis. As triglyceride resulted the VLDL synthesis. As triglyceride levels considerably reduced by this extraction, we can expect the VLDL synthesis reduction.

Cholesterol increases after Alloxan injection is according to Moorthy, Yadaw, Bequer surveys (20) and extraction injection can reduce the cholesterol levels that are according to Amarlaj findings (2). In the diabetic rats we can saw the increased LDL level and reduces HDL levels that it is according to Abou-Seif, MA Ishla, Bhartnagar, Winocour Durrington and Yussef AA findings (1, 18). Cucurbita leaf hydro-alcohol extraction can reduced the LDL levels and increased the HDL levels. Because VLDL indirectly involved in LDL particles production, so that the increase of VLDL in plasma resulted in LDL increase.

As this extraction resulted in considerable VLDL reduction, so that the levels LDL reduced too. Because concentration of HDL in plasma has reverse relation with triglyceride, and Cucurbita leaf could reduce the triglyceride level, so we expected that increased HDL with triglyceride reduction. With recovery in the glucose metabolism pathway, proteins metabolism tend to anabolic pathway instead of catabolic pathway, as result the APO-A1 proteins synthesis, that produce 70% of HDL structure, increased so that HDL levels increase in rats(6).

## CONCLUSION

According to findings we conclude that Cucurbita leaf can use as a drug for reduce of glucose and lipid in diabetic individuals. Meanwhile the HDL level reduction in diabetics individuals is a risk factor for cardiovascular diseases and per 0.1 m mol/lit reduction in HDL level, 1.5 fold increase the possibility of cardiovascular diseases (12), so that perhaps we can say that the forenamed extraction can reduce the possibility of cardiovascular diseases that certainly other surveys are needed.

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