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Do Olive Leaves (*Olea europaea)* have the Ability to Lower Glucose and Cholesterol Levels in Adult Mice?

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Abstract: ***Diabetes is one of the serious problems that require special treatments and can affect humans lifestyle in addition to the huge financial cost that is being spent to find out a new ways that could help in diabetes treatment. Olive leaf extract, derived from Olive leaves (Olea europaea), was used to investigate the biochemical effect of Olive leaves extract as antidiabetic identifying their activity in the reduction of cholesterol level in mice. Exposure of mice to 16 mg/kg body weight of Olive leaf water extract for two weeks resulted in significant decrease of glucose and cholesterol level of mice. A comparison was made between the action of olive leaves extract (1 mg/ml) and Insulin (1 IU/kg), which is known as one of the famous antidiabetic drug. Results showed that glucose level reached 85 mg/dl, while the cholesterol level declined to 47.6 mg/dL in compared to fluctuated decrease in the glucose and cholesterol level of insulin-treated mice. Results showed that extract doesn't have any toxic effect on treated mice even at dose up to 16 mg/kg. Finally, the present study indicated that Olive leaf extract can be a promising, safe and economical alternative to control diabetes and hyper cholesterol disorders.***

Keywords: Olive leaf, Diabetic, Water extraction, Insulin

1. Introduction

Diabetes is a common metabolic disorder characterized by hyperglycaemia due to abnormal insulin production and response. Diabetes is one of the worldwide problems that require special treatments and can affect humans' lifestyle. Diabetes Mellitus can be considered as a one of the major causes of morbidity and mortality in many countries. World Health Organization predicts that's the estimated number of deaths due to diabetes reached 3.4 million people in the recent years. It is also expected to reach 300 million population by 2025. The estimated global cost of managing diabetic patients in 2010 was $376 billion, representing 12% of global health expenditure. Inspite of diabetic complications, the higher level of cholesterol represents a major indicator of health and cardiovascular problems (1,2).

Olive (Olea europaea) tree is a longevous plant, anciently known in the Mediterranean basin (3). The olive tree has been widely accepted as one of the species with the highest antioxidant activity via its oil, fruits, and leaves. Olive leaves are cheap raw materials and useful source of valued products (4,5) that have medicinal usage for diabetes treatment,, (6) blood pressure, and artherosclerosis (7).

Olive leaf extract (OLE) is a supplement derived from the leaves of the plant that bears olive and contains the main bioactives of hydroxytyrosol/tyrosol and oleuropein/ ligstroside. Olive leaves have some medicinal history mostly toward being anti-diabetic and cardioprotective in addition to it's known effect in protecting pancrease from autoimmune damage. In a study conducted in rats, chemically induced type I diabetes noting that olive leaf ingestion (100mg/kg; 19.8% oleuropein) was able to prevent the diabetogenic consequence of low-dose streptozotocin injections and cyclophosphamide (8).

In this study we determined the effect of the simple water extract of olive leaveson glucose and cholesterol levels in adultmice in comparison with insulin. The aim of this study is to evaluate the extract usage as an effective, economical anti-diabetic with less or no side affects that caused by other synthesized drugs.

2. Materials and Methods

Olive leaf collection and extraction

Plant leaves of *Olea europaea* were collected during offspring/2015.Water extraction was done to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional 'herbal' drug (9). Extraction process of olive leaves was achieved according to Sukhdev *et. al,* 2008. The basic operation included many steps, such as pre-washing, drying of plant materials, grinding to obtain a homogenous sample. 50 grams of grinded Olive leaf was soaked in250 ml of boiling distilled water for one hour. The mixture was filtered through with filter paper, and poured in agar plate to be dried using incubator at 40 $^{\circ}$C for 24 hours (10).

Animals:

Eighteen mice weighing around 25g were obtained from the Animal House of the Biotechnological Research Center of Al-Nahrain University, Iraq, Baghdad. Mice were adapted to the experimental laboratory by adjusting the temperature at 20^{o} C, and 12:12 light: dark cycle. Mice were housed in standard plastic cages, fed with standard diet and water. Mice were divided into three groups of six mice in each, and then subjected to one of the following treatments:

A- Group (1): 6 mice used as a control treated only with distilled water.

B- Group (2): 6 mice treated with 1 mg/ml olive leaf extract.

C-Group (3): 6 mice treated with IU/kg of insulin subcutaneously.

After (14) days of treatment, blood samples were collected for determination of plasma glucose and cholesterol levels after two hours of treatment.

Drug preparation and administration:-

Preparation of Olive leaves extract for oral injection:-

Olive leaf extract was orally gavaged, with a concentration of 1 mg/ml according to mice body weight (25g).The given dose was 0.5 ml/day through oral gavage for 14 days.

Preparation of Insulin dose for oral injection:-

Insulin was used as a comparative factor to determine the effect of olive leaves extract on mice, with a concentration of 1IU/ml of saline. Animals injected with 1µl of insulin solution/1gram of body weight to ensure that the get the dose of 1IU/kg for 14 days.

Biochemical test:

Determination of glucose level:

The blood was collected through heart injection, then glucose level was electronically determined by (Accu Check, Germany).

Determination of cholesterol level:

Cholesterol is measured enzymatically in serum in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol, as follows:

1) Blood was collected by heart injection into a glass tube.
2) Blood stands for 45 min at room temperature to allow complete clotting as the shorter period may results in incomplete clotting and secondary clots may formlater.
3) (3)Samples were centrifuged at 1,500 x g for 30 min at 4°C using refrigerated centrifuge, then samples placed into an ice bath immediately after centrifuging and maintained at 2-4° C thereafter.
4) Electronic determination of cholesterol level was achieved directly using – instrument. Desirable cholesterol levels are considered to be those below 200 mg/dL in adults and below 170 mg/dL in children (11).

3. Results and Discussion

The present study investigates the activity of OLE as anti-diabetic and cholesterol controller in treated mice for 14 days compared with usage of insulin, as a way to use as drugs to diabetic patients.

Results showed that water extract of olive leaves, which represents one of the traditional methods of treatment, have a potential impact on mice blood glucose level in addition to their effect to regulate its blood cholesterol level. The usage of Olive leaves aimed to test the natural medicinal plants has lesser side effects when compared with synthetic drugs.

Figure (1) demonstrates that OLE group showed a significant decrease in the level of blood glucose in comparison with control mice. It is also shown that maximum glucose level reach 97 mg/dl in treated mice, compared to 159 mg/dl as a maximum glucose level in control mice. On the other hand, results showed that glucose level was at range of 85-97 mg/dl after treatment.

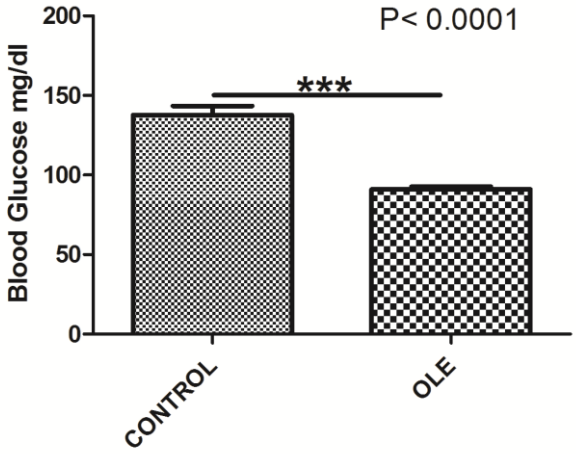

Figure 1: Effect of Olive leaf extract on blood glucose level (mg/dl) in adult mice: Histograms showing the blood glucose levels in control group and OLE group after 14 days of treatment. N=6, P value is 0.0001. T.Test analysis.

Figure (2), indicated a significant P<0.01decrease in blood glucose in OLE (after 14 days)and insulin groups (after two hours of injection) in comparison with non-treated control animals group. There was no sign

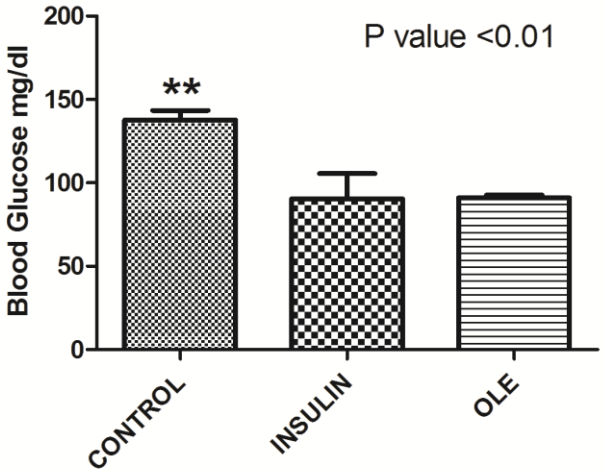

Figure 2: Effect of Insulin on Blood Glucose level (mg/dl) in adult mice

Histograms show the effect of OLE and insulin on basal blood glucose level in comparison with control (non-treated animals). N=5-6. P<0.01). ANOVA one way analysis. Standard error bar in OLE group was more tighten in comparison with insulin group meaning that OLE group was more homogenous than insulin group.

Lakshmi, R. K. et al.,2016 reported that's Olive is one of the medicinal plants that's have hypoglycemic activity used to cure diabetes mellitus, which is a metabolic disorder caused due to improper metabolism of proteins, carbohydrates and fats. It is suggested that the major cause of this disorder is insufficient production of insulin by pancreas or improper utilization of produced insulin in the body or due to destruction of beta cells in the pancreas (12).

Therefore, in spite of the availability of synthetic drugs, which includes insulin and glibenclamide in the markets as therapeutic drugs, the OLE could have potential hypoglycemic activity with fewer side effects, besides their availability, simplicity and economical cost.

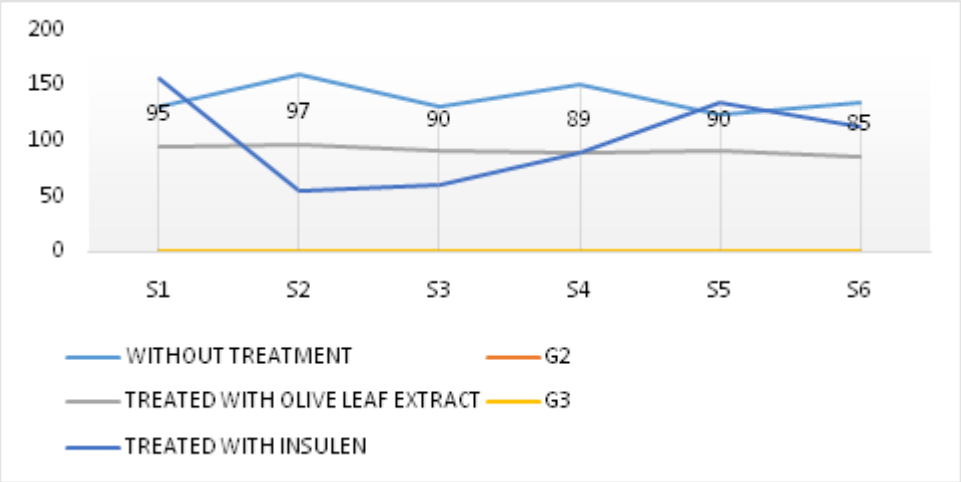

Figure 3: Comparative evaluation of the blood glucose level (mg/d) among mice groups

This figure shows the fluctuation in levels of blood glucose among control, insulin-injected, and OLE groups. OLE shows the best distribution of of blood glucose values within the group (gray line).

The antidiabetic effect of OLE suggested to be due to their bioactive compounds, mainly oleuropein, which is a highly pungent compound claimed to be the cause of olive oil's distinct taste. In spite of the activity of bioflavanoid rutin, luteon, and hesperidin, which work together with oleuropein to enhance its natural activity. Oleuropein showed effective effect on the blood sugar levels, by working independently oninsulin to stimulate glucose uptake. This effect is believed to be related with the glucose-induced insulin release (13, 14).

Wainstein J. et al., (2012) showed that OLE that's orally administered to human and rat may represent an effective adjunct therapy that normalizes glucose homeostasis in individuals with diabetes (15).

On the other hand, the effect of OLE on cholesterol level were estimated in the same comparison way with control mice and insulin treated mice, results showed in figure (4) indicated significant decline in the level of cholesterol mice treated with OLE in comparison to control mice. However, Insulin treated mice indicated relative level of cholesterol in their blood in comparison to control mice.

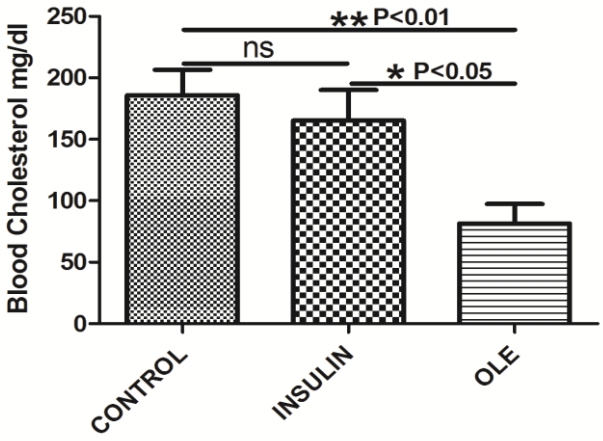

Figure 4: Effect of Olive leaves extract on Blood Cholesterol level (mg/dL) in adult mice

Histograms showing the effect of insulin and OLE injection on blood cholesterol level. Insulin did not change the level of cholesterol level in comparison with control group. OLE group showed a significant decrease in cholesterol level **P<0.01 with control group and *P<0.05 with insulin-injected group. N=6. ANOVA one way analysis.

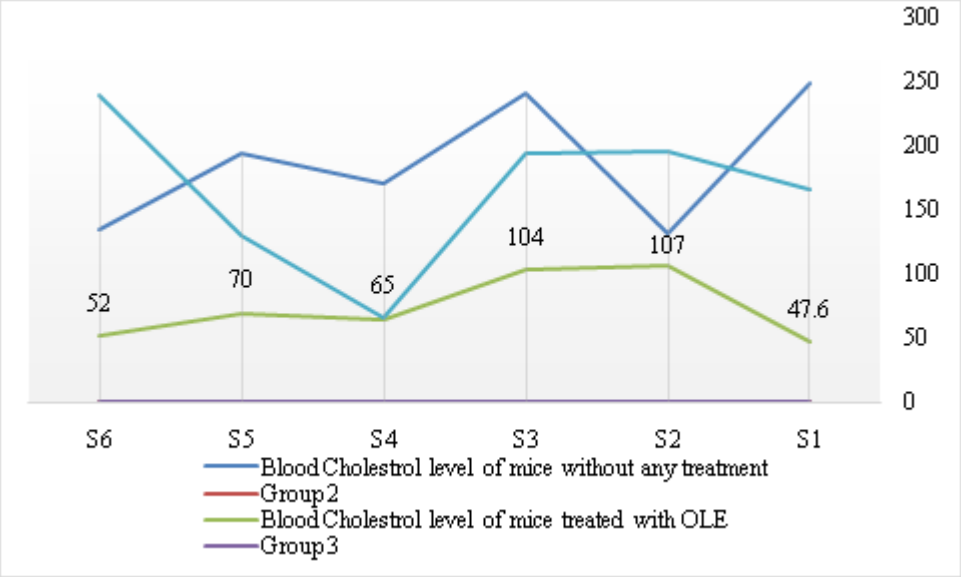

Figure 6: Comparative evaluation of the Cholesterol level (mg/dL) among mice treated with OLE, Insulin and untreated mice

The comparative view of the effect of OLE and insulin on treated mice showed that OLE had maximum and stable effect on cholesterol level of mice blood than that determined in insulin treated mice. These data ensure OLE ability to control the level of cholesterol at the normal ranges. Animal studies using rats prone to artherosclerosis and high cholesterol have noted reducing protective effects of olive leaf extract (16).

In spite that OLE never showed any toxicity activity on mice during the study, which suggest that OLE could be a safe promising therapeutic drugs of the treatment of diabetic, cholesterol and their cardiovascular complications. Studies on olive leaf extract have failed to notice any significant side-effects when doses up to 1000 mg daily for 8 weeks or lower doses (7, 17).

In addition, the physical investigation was done by Atefand Isam (2014), showed that the supplementation with combination of olive and tea leaves extract led to more attenuation effect against diazinon toxicity, one of the toxic organophosphorous insecticides, in male mice (18).

Its also reported that Olive and rosemary leaves extract showed antioxidant activity against the toxic thioacetamide compounds (19).

In conclusion, we conducted this study to figure out some new methods that use natural plants to help out in reducing blood glucose and cholesterol levels in diabetic individuals. Even though this study was performed and conducted in rodents, it still gives good evidence that olive leaves extract could successfully reduce both of glucose and cholesterol levels in comparison with control group and that might be applicable in diabetic humans.

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