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The hypoglycemic effect of oleanolic acid which extracted from *Alhagi Roots* in diabetic rabbits by improve antioxidant defenses

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ABSTRACT

This research include extractoleanolic acid (OAA) from Alhagi roots was administered orally to diabetic rabbits induced by alloxan once per day for one month. This treatment has been given significant decreasing of blood glucose, lipids and lipid peroxidation levels (malondiadehyde MDA). In addition, the levels of antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD) decreased in diabetic rabbits however these levels returned to normal when treated by oleanolic acid. From this results can suggest the oxidative stress plays important role in diabetes, and treatment with oleanolic acid useful in controlling not only for glucose and lipid levels, but these components may be also improvement the antioxidants potential.

Keywords: oleanolic acid, *Alhagi* , diabetes, extract, Free radicals, oxidants, antioxidants, lipid peroxidation.

INTRODUCTION

Both oxygen free radicals (OFRs) and malondialdehyde (MDA) are main responsible in the pathogenesis of a large number of diseases such as cancer, diabetes, infectious diseases, coronary artery diseases and in aging [1, 2] .

From the mechanisms which contribute to the formation of free radicals in diabetes mellitus include increasing non-enzymic and auto oxidative glycosylation, also the levels of inflammatory mediators, metabolic stress resulting from changes in energy metabolism, and the status of antioxidant defense systems [3]. The evidence of increase the oxidative stress in diabetes due to overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant defenses. Therefore, the oxidative stress, as well as non-enzymic glycosylation, is now considered as a major factor to extent of chronic diabetic complications [4].

Lipid peroxidation and free radicals have been share in many of the criteria required for a role in the pathogenesis of diabetic cases [5]. In contrast, to control on ROS, aerobic cells must be developed their own defense antioxidant systems, these system, based on enzymic control which includes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [7,6].

In the past, herbs and medical plants were being used for the treatment of various diseases around the world. where as many herbal products have been described for diabetes mellitus. The anti-diabetic properties of some chemical compounds like oleanolic acid which extracted from *Alhagi roots* in Iraq and abundance in nature can consider from encouraging factors to use this herb as a source to produce oleanolic acid which can use as a dietary supplements for lowering the blood glucose level in diabetic patients [8-10].

However, Oleanolic acid and ursolic acid (Fig. 1) are the main active components in Alhagi, and have many medical uses useful such as anticancer, antimutagenic, anti-inflammatory,antioxidative activities. oleanolic acid is a

terpenoid compound isolated from Alhagi roots. and from the other beneficial potentials in nutrition, cosmetics and drugs.

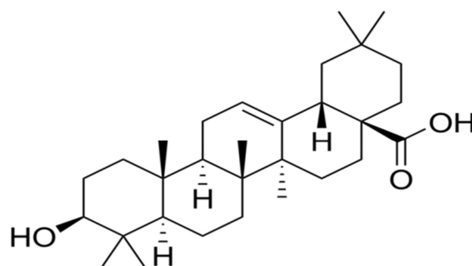

Figure 1: Oleanolic acid (OAA)

The purpose of this study to investigate the protective effect for oleanolic acid in lowering the blood glucose level, lipid peroxidation, serum lipids and antioxidant enzymes status in alloxan induced diabetic rats.

MATERIALS AND METHODS

General procedure for plant preparation:
The fresh plant roots of *Alhagi* were collected from Babylon city during July 2015 and stored immediately after collection at 8°C. The samples roots were cleaned and cut to small pieces for grinding. Weight of 15 g of powdered roots were extracted with ethanol by using Soxhlet extraction apparatus for about 30 mins [11].

The crude extract was concentrated under vacuum by using lympholizer apparatus (Freeze dryer) to produce about (2.4 g, 16%) of crude extract.

General procedure of isolation product The same crude extract directly packed into flash column chromatography (FCC) with the following descriptions (Column size 30 × 500 mm, packed with stationary phase silica gel 220-400 mesh size. The mobile phase used (30 : 70, dichloromethane : hexane) [11].

Experimental Animals:
The experimental animals were treated as following:

Rabbits housing:
Thirty nine male rabbits (1000-1400) gram body weight and (3-5) months of age were purchased from local market/Babylon/Hilla.

The rabbits housed under controlled animal conditions of temperature (25±3 C°) and relative humidity (50±5)%.

Upon arrival, animals were adapted for three weeks and were maintained on a regular feed (control diet). It is known by pelleted (Crude protein 10%, ground soybean 20%, wheat flour 35%, corn 35%, mineral & vitamins 1 gm/Kg). Total energy was 13.6 KJ/Kg protein.

For determine the active dose of oleanolic acidby depended on other research and its 100 mg/kg.

Diabetes Mellitus Induction:
The rabbits were injected intravenous with alloxan mono-hydrate [(2,4,5, 6) tetraoxyhexa hydro pyrimidine] to induce the diabetes after fasted 12 hrs. The compound was freshly prepared (150 mg/Kg) dissolved in 1ml of normal saline, and given as a single dose.

The rabbits have been taken 5% glucose with tap water for the first day only after dose of alloxan. Then left to relief and to eat enough after 3 days later the rabbits had diabetes indicated the blood glucose had more than 200 mg/dL,and appearances on its fatiguing signs such as polyuria and polydipsia.

Animals and experimental design: The rabbits were divided into three groups:

Table 1: The categories of experimental animals according to treatment

Treatment with	The Groups	No. of rabbits	Dose of OAA (mg/Kg)
Control	Group A	20	------
Without treatment	Group B	20	------
With treatment	Group C	20	100

Were given orally at the same time for one month.

Blood sampling: Fasting blood samples were collected from marginal ear vein by using insulin syringe (1ml) (G27×1/2") and capillaries once every day (for each experiment) for determine the glucose levels and syringing the blood from the hart after 1 month by syringe (5ml) (G23×1"). Blood was allowed to clot 10 minutes then centrifuged at (4000 rpm=0.894Xg) for 10 minutes.

Determination of Malondialdehydaae (MDA):
The principle of the following method was based on the spectrophotometric measurement of the color, occurred during the reaction between thiobarbituric acid (TBA) and MDA,[12].

Determination of reduced glutathione (GSH):
More than one type of analytical methods are used to determine serum glutathione (GSH) depending on the action of sulfhydryl groups. Thus methods include photometric, enzymatic, flourometric and HPLC are used [13].

Determination of Superoxide dismutase activity (SOD):
Pyrogallol (1,2,3-benzenetriol) has long been known to autoidize rapidly, especially in alkaline solution and the reaction has been employed for the removal of oxygen from gases [14].

Determination of Catalase (CAT):
The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured calorimetrically at 570-610 nm [15].

Triglyceride Determination (TG):
Triglyceride in the serum was measured enzymatically [16].

Total cholesterol Determination:
This method for the measurement of total cholesterol in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine due proportional to the concentration of cholesterol in sample [17].

High-density lipoprotein (HDL):
High density lipoprotein concentration was measured enzymatically which based on the fact that LDL and VLDL precipitated with phosphatuntic acid in the presence of magnesium ions at room temperature, while HDL remains in the supernatant [18].

Determination of Blood-Glucose:
The principle of the measurement is based on Trinder reaction: glucose is oxidized to D-gluconate in the presence of glucose oxidase (GOD) with hydrogen peroxide formation [18].

RESULTS

The following table illustrated the biochemical parameters before and after treatment by OAA, when induced diabetes in rabbits by alloxan.

Table 2: Biochemical variable in each group A,B, and C

ParameterBiochemical	Group A	Group B	Group C
GSH (µmol/L)	30.28±3.68*	9.08±2.55**	26.94±3.93
SOD (IU/L)	5.44±0.78*	2.77±0.57**	4.16±0.56
CAT (IU/L)	82.08±4.14*	55.46±8.90**	71.14±5.65
MDA (µmol/L)	1.44±0.02*	5.24±0.62**	1.83±0.24
Blood Glucose (mg/dl)	98±7.1*	260±19**	165±16***
Triglyceride (mg/dl)	121.12±9.62*	170.54±12.16**	128.52±9.40***
Total Cholesterol (mg/dl)	159.36±5.67*	187.66±10.17**	165.47±9.60
HDL-Cholesterol (mg/dl)	61.26±3.16*	43.70±4.91**	54.36±3.25***
LDL-Cholesterol (mg/dl)	68.44±8.20*	116.15±10.59**	76.52±7.91***
VLDL-Cholesterol (mg/dl)	24.25±1.26*	31.24±2.61**	26.71±1.66

Values are given as mean ± S.D from 20 subjects in each group.
** rabbits without treatment compared with control, subjects (p<0.05).** rabbits without treatment compared with treatment rabbits, subjects (p<0.05).*** rabbits with treatment compared with control, subjects (p<0.05).*

DISCUSSION

Alloxan has been doing partial destruction of β-cells, by using dosage (150 mg/kg) intravenous. This process lead to occur type 2 diabetes for rabbits (Table 2) by increasing blood sugar more than normal values. At the same time, this destruction of Langerhans islets by alloxan completely by producing free radical.

Through the results in the (Table 2), can note the presence of a significant decrease in the values of GSH, SOD, CAT for groupB compared with group A. By comparison, existence of significant rise in the value of MDA of group B compared with group A. This may be due to the rise in free radicals to higher levels than in the control group (Group A).

To improve the interpretation of antioxidants, the treatment by using of OAA to do on raising the values ofenzymatic and non-enzymatic. Because of OAA is one of family tannin compounds.

The efforts to reduce the oxidative stress has an important role in the rebuilding of beta cells of pancreas. So, note down the sugar levels of the (group C) when a one-month treatment

When we returned to results of lipids (Table 2), the elevated of serum cholesterol, triglyceride, LDL-cholesterol, VLDL-cholesterol, and lowering values of HDL-cholesterol of rabbits without treatment (group B). This is may be due to lowering efficiency of hepatocellular because increase the values of lipids peroxidation (MDA). In contrast, the causes of decrease in the values of LDL-cholesterol after treatment by OAA (group C) may be due to activated macrophages to release reactive oxygen species ROSs,scavenge oxidized low-density lipoprotein (oxLDL), becomefoam cells, and lead to the development of the fatty streak in the early stage of atherosclerosis [19,20].

Finally, the antioxidant properties of OAA, due to chemical formula in nature. It is consider polyphenol compound because contain double bond and unconjugated system, and this make as scavenger for free radical.

CONCLUSION

In briefly, the medical herbs have large size in pharmacy nowadays, because its more safety and easier in preparation than drugs.

The protective effect of OAAagainst cardiovascular disease and cancer. And its beneficial properties for enhanced antioxidant defense, lowering of blood lipids, and blood sugar. So, Its consider as important element in the field of medicine.

In the end, *Alhagi* from more abundant herbs in Iraq. Therefore, we have to concentrate more to take advantage of this herbs in medicines industry.

REFERENCES

[1] Akkus I, Kalak S, Vural H et al., *ClinChimActa* **1996**; 244: 221–227.
[2] Yaki K, *Bio Essays* **1984**; 1: 58–60.
[3] Griesmacher A, Kindhauser M, Andert SE etal., *Am J Med* **1995**; 98: 469–475.
[4] Giron MD, Salto R, Gonzalez Y et al., *Chemosphere* **1999**; 38: 3003–3013.

[5] Gul M, Kutay FZ, Temocin S, Hanninen O, Cellularand clinical implications of glutathione. Ind J ExpBiol **2000**; 38: 625–634.
[6] Michiels C, Raes M, Toussaint O, Ramacle, *Free Rad Biol Med* **1994**;17: 235–248.
[7] Alarcom-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S et al., *J Ethnopharmacol* **1998**; 61: 101–110.
[8] Khosla P, Sangeeta B, Singh J, Seth S, SrivastavaRK. *Ind J PhysiolPharmacol* **2000**; 44(1): 69–74.
[9] Anu Chandra, Singh RK, Singh VK, Tewari L,Mahdi AA. *SFRR-India Bulletin* **2004**; 3(1): 24–26.
[10] Elder C. *AlternTher HealthMed* **2004**; 10(1): 44–50.
[11] Alaa J. Mahrath, Ghafil S. Hassana And Ehab K. Obaidb*, Int. J, Chem. Sci*.: 13(2), **2015**, 983-989
[12] Sharma V. K., Kumar S., Patel H. J. and Hugar S., "Hypoglycemic activity of ficus*Glomerata* inalloxan induced diabetic rats",*International Journal of Pharmaceuticaal Science Review and Research*; **2010**, 1(2):18-22.
[13] Monica Cheesbrough, " District Laboratory Practice in Tropical Countries", Part 1, 2nd ed., United States of America by Cambridge University Press, New York, (**2006**); P: (299-318).
[14] Burtis C.A. and Ashwood E.R. Tietz, "Textbook of clinical chemistry", 3rd ed., W.B. Saunders Company, Tokyo, (**1999**); P: (1034-1054).
[15] Stryer L. "Biochemistry". 4th ed., New York, Freeman W. H. and Company. **2000**) (Ellman G. L, "Tissues sulfhydryl group". Arch. Biochem. Biophys. (**1959**) ; 82:70-77)(Sedlak J., Lindsay R. H. "Anal. BIOCHEM.". **1968**;25:192).
[16] Marklund S., and Marklund G, *Eur. J. Biochem*. (**1974**); 47:469–474) .
[17] Sinha A.K, *Anal Biochem.* (**1972**); 47: 389–394).
[18] Young DS, Effects of drugs on clinical laboratory test. 4th ed. (**1995**).
[19] Young DS, Effects of drugs on clinical laboratory test. 5thed, (**2000**).
[20] Tietz, **1995**)Tietz. NW,(Clinical Guide to Laboratory Tests, 3rd ed. W.B.Sanuders Co. Philadelphia PA), (**1995**).