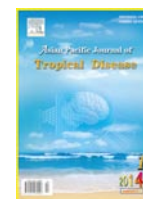




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## Hypoglycemic, hypolipidemic and antiatherogenic effects of oleuropein in alloxan-induced Type 1 diabetic rats

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### PEER REVIEW

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#### Comments

This is a good study in which the authors showed that oleuropein has beneficial effects, in decreasing the elevated hemoglobin A1C, serum glucose, lipid profile, and atherogenic index in alloxan-induced-diabetic rats. Details on Page S424

### ABSTRACT

**Objective:** To assess effect of oleuropein on hemoglobin A1C, serum glucose, lipid profile and atherogenic index in alloxan-induced Type 1 diabetic rats.

**Methods:** Thirty Sprague–Dawley male rats were divided into three groups randomly; group one as control, group two diabetic untreated, and group three treatments with oleuropein by 15 mg/kg *i.p.* daily, respectively. Diabetes was induced in the second and third groups by alloxan injection subcutaneously. After 8 weeks, the levels of hemoglobin A1C, fasting blood glucose, triglyceride, cholesterol, low density lipoprotein, very low density lipoprotein, high density lipoprotein and atherogenic index of all groups were analyzed.

**Results:** Oleuropein significantly decreased hemoglobin A1C, fasting blood glucose, triglyceride, cholesterol, low density lipoprotein, very low density lipoprotein. High density lipoprotein level was significantly increased when treated with oleuropein.

**Conclusions:** The findings of the present study suggest that oleuropein exert beneficial effects on serum glucose, hemoglobin A1C, lipid profile and atherogenic index in alloxan-induced Type 1 diabetic rats.

### KEYWORDS

Oleuropein, Diabetes, Lipid profile, Atherogenic index, Hemoglobin A1C, Rat

## 1. Introduction

Hyperglycemia is confounded for the complications of diabetes because hyperglycemia directly causes glycation of proteins, lipids and nucleic acid then injures cells and induces lipid peroxidation[1]. Also antioxidant and antioxidative enzyme activities reduce due to glycation or increase of lipid peroxidation products[2,3]. A number of natural antioxidant such as vitamin E and phenolic compounds are known to have hypoglycemic, hypolipidemic

or both activities[4]. Chemical drugs have many side effects; therefore, screening for new antidiabetic sources from natural antioxidants is still attractive because they are safe and good alternative for treatment of diabetes mellitus. A growing body of research indicates that nutritional deficiencies such as antioxidants contribute to the development of diabetes. Oleuropein is a secoiridoid derived from olive leaf and olive oil[5]. Several studies have demonstrated that oleuropein has a high antioxidant activity[6]. Oleuropein inhibits low-density lipoproteins

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(LDL) oxidation *in vitro* and lipid peroxidation *in vivo* and scavenges free radicals[7], hypochlorous acid-derived radicals, hydroxyl radicals and superoxide anions. Oleuropein has high antioxidant activity *in vitro*, comparable to a hydrosoluble analogue of tocopherol[5]. Previous our study showed that oleuropein has neuroprotective effect in spinal cord injury and protective effect in oxidative spinal cord injury[8].

Since the hypolipidemic, antiatherogenic and protective effects of oleuropein on hyperglycemia and hemoglobin A1C status in alloxan-induced Type 1 diabetic rats have not previously been reported; the objectives of the present study were to investigate hypoglycemic, hypolipidemic and antiatherogenic effects of oleuropein in alloxan-induced Type 1 diabetic rats.

## 2. Materials and methods

### 2.1. Animals

Thirty male mature Sprague–Dawley rats (180–200 g) were obtained from Pasteur Institute of Tehran and were allowed to adapt themselves with the new location for one week. This study was approved by the Animal Ethics Committee of the Medical University of Lorestan with accordance to the national health and medical research council guidelines. The rats were divided to three groups (10 per each). The studied groups were as follows: group 1 as control, group 2 as diabetic without treatment and 3rd group as diabetic treatment with oleuropein.

### 2.2. Diabetes induction

Diabetes was induced after overnight fasting in the second and third groups by injection of alloxan monohydrate (120 mg/kg) subcutaneously[9]. Beta cell degradation by alloxan leads to release of more insulin. Because of acute hypoglycemia, the rats received 10% sucrose solution for 48 h instead of drinking water. Five d after induction of diabetes, blood samples were gathered from the end part of tails. Blood glucose was measured by glucometer and the rats with blood glucose level of  $\geq 300$  mg/dL (16.7 mmol/L) were considered as diabetic[10]. During the first 5 d after diabetes induction, 1–3 rats per group died because of alloxan toxicity. The rats were kept at 12/12 dark–light period in  $(21 \pm 3)$  °C temperature. All animals were allowed free access to food and water *ad libitum* during the experiment. The third group was treated with oleuropein by 15 mg/kg *i.p.* daily[10]. The treatment was begun at the first day of diabetes induction. After 8 weeks treatment, animals were anesthetized (Nesdonal 50 mg/kg, *i.p.*), blood samples were obtained from hearts and allowed to clot for 20 min in laboratory temperature and then

centrifuged at 3000 r/min for 10 min for serum separation[9].

### 2.3. Biochemical study

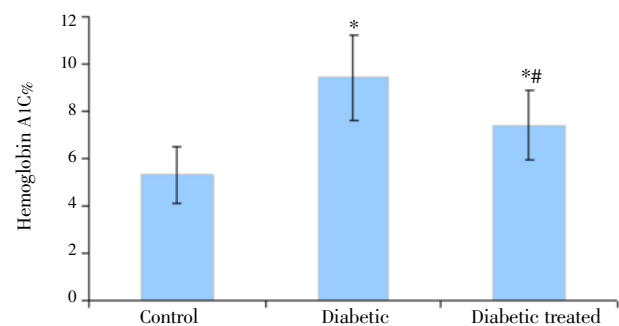
The serum levels of fasting blood glucose, triglyceride (TG), cholesterol, LDL, very low density lipoprotein (VLDL), high density lipoprotein (HDL), and atherogenic index of all groups were analyzed. Fasting blood glucose, cholesterol and TG concentrations were measured by biochemical analyzer using commercial kits (Olympus AU–600, Tokyo, Japan). HDL was measured in the supernatant after the precipitation of the Apo–B containing lipoproteins (LDL and VLDL) using polyanions in the presence of a divalent cation[11]. LDL and VLDL were determined by calculation using the Freidewald *et al.* equation[12]. Also the hemoglobin A1C was determined using a hemoglobin A1C assay kit (Randox Lab., Ltd., UK) according to the manufacturer's protocol. The atherogenic index was determined by calculation using the Ikewuchi and Ikewuchi equation[13].

### 2.4. Statistical analysis

All values are expressed as mean  $\pm$  SEM. The data were compared between groups by Mann–Whitney U test. Statistical analyses were performed using the SPSS 13 for windows software. A *P* value of  $< 0.05$  was considered statistically significant.

## 3. Results

The level of hemoglobin A1C in the untreated diabetic rats was significantly (1.61-fold) higher than that of control animals. The treatment of diabetic animal with oleuropein could significantly (23%) inhibit the increase of hemoglobin A1C in comparison with the untreated diabetic animals (Figure 1).

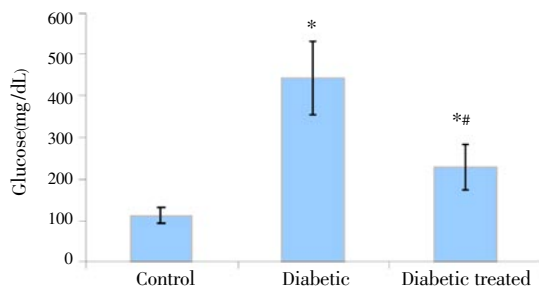


**Figure 1.** The effect of oleuropein on hemoglobin A1C in alloxan induced diabetic rats.

\**P* < 0.05 as compared with control group. \*\**P* < 0.05 as compared with diabetic without treatment group.

The level of glucose in the untreated diabetic rats was significantly (4.81-fold) higher than that of control animals. The treatment of diabetic animal with oleuropein

could significantly (29%) inhibit the increase of glucose in comparison with the untreated diabetic animals (Figure 2).



**Figure 2.** The effect of oleuropein on serum glucose in alloxan induced diabetic rats.

\* $P < 0.05$  as compared with control group. # $P < 0.05$  as compared with diabetic without treatment group.

The level of total cholesterol (TC) in the untreated diabetic rats was significantly (1.54-fold) higher than that of control animals. The treatment of diabetic animal with oleuropein could significantly (24.80%) inhibit the increase of cholesterol in comparison with the untreated diabetic animals (Table 1). The level of TG in the untreated diabetic rats was significantly (1.53-fold) higher than that of control animals. The treatment of diabetic animal with oleuropein could significantly (22.82%) inhibit the increase of TG in comparison with the untreated diabetic animals (Table 1).

**Table 1**

Effect of oleuropein on TG, TC, LDL, HDL, VLDL and atherogenic index in diabetic rats.

Parameter	Control	Diabetic	Diabetic+Oleuropein
TG (mg/dL)	82.33±14.75*	126.57±18.59	97.69±13.91*
TC (mg/dL)	72.01±16.35*	110.88±28.48	83.38±20.75*
HDL (mg/dL)	39.00±13.29*	25.01±9.19	38.13±10.67*
LDL (mg/dL)	26.52±21.58*	60.57±28.18	25.73±21.06*
VLDL (mg/dL)	16.47±2.49*	25.30±3.44	19.55±2.78*#

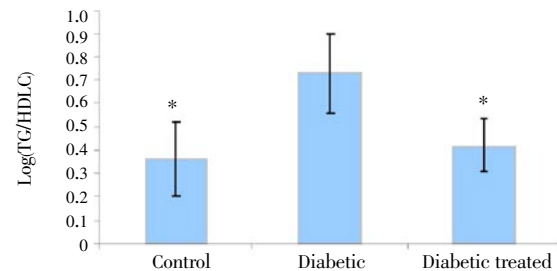
Values are represented as Mean±SD oleuropein: \*Significant change in comparison with diabetic without treatment at  $P < 0.05$ . #Significant change in comparison with control at  $P < 0.05$ .

The level of LDL in the untreated diabetic rats was significantly (2.28-fold) higher than that of control animals. The treatment of diabetic animal with oleuropein could significantly (57.52%) inhibit the increase of LDL in comparison with the untreated diabetic animals (Table 1). The level of VLDL in the untreated diabetic rats was significantly (1.54-fold) higher than that of control animals. The treatment of diabetic animal with oleuropein could significantly (22.73%) inhibit the increase of VLDL in comparison with the untreated diabetic animals (Table 1).

The level of HDL in the untreated diabetic rats was significantly (1.56-fold) lower than that of control animals. The treatment of diabetic animal with oleuropein could significantly (34.41%) increase of HDL in comparison with the untreated diabetic animals (Table 1).

The level of atherogenic index (units) [ $\log(\text{TC}/\text{low-density lipoprotein cholesterol (HDL-C)})$ ] in the untreated diabetic rats was significantly (2.03-fold) higher than that

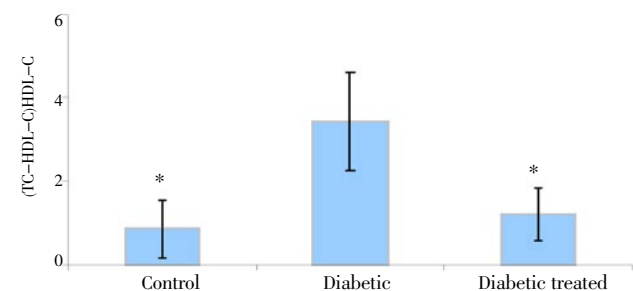
of control animals. The treatment of diabetic animal with oleuropein could significantly (42.46%) inhibit the increase of atherogenic index in comparison with the untreated diabetic animals (Figure 3).



**Figure 3.** The effect of oleuropein on Atherogenic index (units)  $\log(\text{TC}/\text{HDL-C})$  in alloxan induced diabetic rats.

\* $P < 0.05$  as compared with diabetic group.

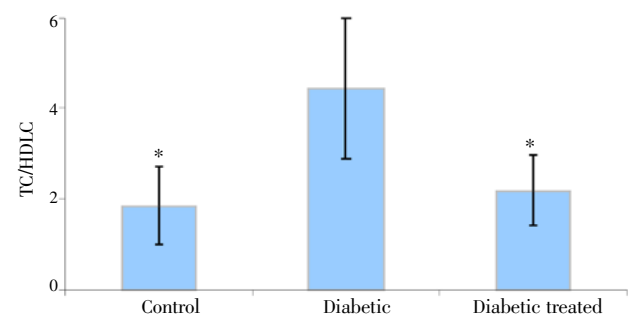
The level of atherogenic coefficient  $[(\text{TC}-\text{HDL-C})/\text{HDL-C}]$  in the untreated diabetic rats was significantly (3.78-fold) higher than that of control animals. The treatment of diabetic animal with oleuropein could significantly (65.99%) inhibit the increase of atherogenic coefficient in comparison with the untreated diabetic animals (Figure 4).



**Figure 4.** The effect of oleuropein on atherogenic coefficient  $((\text{TC}-\text{HDL-C})/\text{HDL-C})$  in alloxan induced diabetic rats.

\* $P < 0.05$  as compared with diabetic group.

The level of cardiac risk ratio (TC/HDL-C) in the untreated diabetic rats was significantly (2.42-fold) higher than that of control animals. The treatment of diabetic animal with oleuropein could significantly (52.71%) inhibit the increase of cardiac risk ratio (TC/HDL-C) in comparison with the untreated diabetic animals (Figure 5).

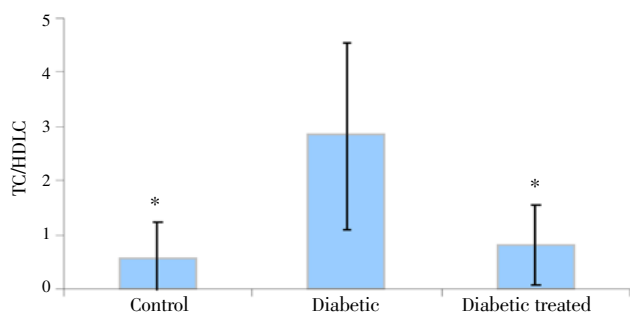


**Figure 5.** The effect of oleuropein on cardiac risk ratio (TC/HDL-C) in alloxan induced diabetic rats.

\* $P < 0.05$  as compared with diabetic group.

The level of cardiac risk ratio (LDL/HDL-C) in the untreated diabetic rats was significantly (4.88-fold) higher than that of control animals. The treatment of diabetic animal with

oleuropein could significantly (71.73%) inhibit the increase of cardiac risk ratio (LDL/HDL-C) in comparison with the untreated diabetic animals (Figure 6).



**Figure 6.** The effect of oleuropein on cardiac risk ratio (LDL/HDL-C) in alloxan induced diabetic rats.

\* $P < 0.05$  as compared with diabetic group.

#### 4. Discussion

Diabetes significantly increased hemoglobin A1C and serum glucose, TG, cholesterol, VLDL and LDL concentrations in comparison with the control group. Treatment of diabetic animals with oleuropein significantly inhibited increase of hemoglobin A1C and serum glucose, TG, cholesterol, VLDL and LDL concentrations and atherogenic index in comparison with the untreated diabetic animals. Also, researchers showed that treatment of diabetic animals with oleuropein could reduce serum TG, cholesterol and uric acid level in myocardial injury induced by ischemia and reperfusion[14]. Also, researchers showed oleuropein could reduce serum TG, cholesterol and free fatty acid concentrations in hypercholesterolemic rabbits[15]. Moreover, researchers showed oleuropein from olive leaves has antidiabetic and antioxidant effects[16]. Also researchers showed that the addition of 10% (w/w) extra virgin olive oil and 7 mg/kg oleuropein to the standard diet reduces plasma levels of total cholesterol[14,17].

Results of our study are in accordance with other researchers' study that showed oleuropein similar to others antioxidants such as vitamin E and coenzyme Q10 could reduce hemoglobin A1C and prevent hyperglycemia[18]. Therefore natural antioxidant with hypoglycemic, hypolipidemic and antiatherogenic effects could prevent or be helpful in reducing the complications of hyperglycemia and hyperlipidemic seen in diabetes patients. The mechanism of hypoglycemic, hypolipidemic and antiatherogenic action of natural antioxidant may be due to the inhibition of dietary lipid absorption in the intestine or its production by liver or stimulation of the biliary secretion of cholesterol and cholesterol excretion in the feces[19,20]. Also, the mechanism of hypolipidemic and antiatherogenic action of natural antioxidant may be due to the inhibition of glycation lipoproteins, enzymes and proteins that involve lipid and lipoprotein metabolism[21,22]. Also, the mechanism

of hypoglycemic effect of natural antioxidant may be due to the potentiation of glucose induced insulin release, increase peripheral uptake of glucose and attenuating oxidative stress and enhancing of body's own antioxidant defenses[23]. Although, the detailed molecular protective mechanisms of oleuropein can not be fully explained by our results, our results are satisfactory. Oleuropein as a natural antioxidant with multi beneficial properties can be introduced to diabetic patients without diabetic nephropathy for inhibition of progression of diabetic.

This study showed that oleuropein has beneficial effects, in decreasing the elevated hemoglobin A1C and serum glucose, lipid profile, atherogenic index in alloxan-induced-diabetic rats. Hence, attenuation of hyperglycemia, lipid profile and atherogenic index can decrease diabetic complication such as nephropathy in diabetic patients.

#### Conflict of interest statement

The authors declare that there are no conflicts of interest.

#### Acknowledgements

The authors wish to thank Deputy of Research and Razi Herbal Research Center of Lorestan Medical University, Lorestan. Iran, Grant No. 91/21.

#### Comments

##### Background

Hyperglycemia is confounded for the complications of diabetes because hyperglycemia directly causes glycation of proteins, lipids and nucleic acid then injures cells and induces lipid peroxidation. Chemical drugs have many side effects; therefore, screening for new antidiabetic sources from natural antioxidants is still attractive because they are safe and good alternative for treatment of diabetes mellitus. Oleuropein is a potent antioxidant.

##### Research frontiers

The present study was to assess effect of oleuropein on hemoglobin A1C, serum glucose, lipid profile and atherogenic index in alloxan-induced Type 1 diabetic rats.

##### Related reports

Authors reported neuroprotective effect of oleuropein following spinal cord injury in rats. Also authors reported effect of oleuropein on tissue myeloperoxidase activity in experimental spinal cord trauma. Others authors showed that olive leaves extract is a source of potent antioxidants

and prevents the oxidation of LDL *in vitro*.

### Innovations & breakthroughs

This study showed that oleuropein has beneficial effects, in decreasing the elevated hemoglobin A1C, serum glucose, lipid profile, and atherogenic index in alloxan-induced-diabetic rats.

### Applications

Oleuropein is a potent antioxidant and may be a good alternative to reduce the risk of atherosclerosis and coronary heart disease and diabetic complication such as nephropathy in diabetic patients.

### Peer review

This is a good study in which the authors showed that oleuropein has beneficial effects, in decreasing the elevated hemoglobin A1C, serum glucose, lipid profile, and atherogenic index in alloxan-induced diabetic rats.

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