Original Article

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The protective effects of Olive leaf extract on Type 2 diabetes, the expression of liver superoxide dismutase and total antioxidant capacity of plasma in rats

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Abstract

Olive leaf has medicinal benefits in type 2 diabetes. The aim of this cross-sectional study was to evaluate the effects of the olive leaf extract on the prevention of type 2 diabetes induced by Streptozotocin and nicotinamide, assessment of the expression of liver superoxide dismutase enzyme gene and total antioxidant capacity of plasma in rats. In this study 28 healthy, mature, Sprague-Dawely male rats with the initial weight of 250 ± 50 g were divided into 4 equal groups (group 1: healthy control; group 2: healthy trial; group 3: diabetic control; group 4: diabetic trial). Fasting blood glucose in all rats was measured every week with the glucose oxidase method. At the end of our study, the animals were sacrificed and blood and liver were collected and all needed parameters were measured. Fasting blood glucose was not significantly differente in the group 4 (439±47mg/dl) and group 3 (445±33 mg/dl), but with group 1 (85±11 mg/dl) significant difference was observed. There was no significant difference between total antioxidant activity and nitric oxide metabolites in groups but the expression of superoxide dismutase gene was significantly increased in group 2 and group 3 (P<0.05). Histopathological results of the liver in group 4 showed macrophage accumulation, mild inflammation, apoptosis in comparison with other groups. The extract of olive leaf with a dosage of 100 mg/kg did not reduce blood glucose in diabetic rats. Therefore, traditional methods used to treat diabetes type 1 and 2 do not only prevent but also diminish the ability to reduce blood sugar and can also cause damage to hepatocytes and other complications.

Keywords: Olive leaf extract, Protective effects, Type2 diabetes, Superoxide dismutase, Total antioxidant capacity.

1. Introduction

Type 2 diabetes is defined as elevated blood sugar levels due to impaired insulin secretion, insulin action, or both (1). The illness is associated with microvascular (retinopathy, nephropathy and neuropathy) and macrovascular (cardiovascular) complications (2). In addition to endocrine factors, there are other factors associated with diabetes and its complications (i.e. oxidative stress). Hyperglycemia can induce oxidative stress from different ways; so, oxidative stress plays an important role in cell damage during hyperglycemia. Oxidative stress happens when the immune system is not strong enough to be able to deal with reactive oxygen species (ROS) (3), and reactive nitrogen

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species (RNS) (4). In other words, oxidative stress will appear, when the antioxidant and proxidant decrease and increase, respectively (5). Hyperglycemia causes tissue damage through several different mechanism such as increasing the amount of glucose and other sugars during polyol pathway, activations of protein kinase C (PKC), overactivation of hexose amine pathway, increase in the formation of intracellular advanced glycation end products (AGE), activation of ligands and increasing the expression of AGEs receptors (3). The antioxidant defense system consists of two parts; enzymatic defense system (SOD, CAT, GPX, and GR) and non-enzymatic defense system (vitamin A, C, E) that are important for dealing with reactive species (6). In recent years, a lot of herbal extracts have been used for treatment of diabetes type 2 such as mulberry leaf extract (7), phenolic-rich extract of soybean (8), rosemarinus extract (hypoglycemic and hepatoprotective agent) (9) and cinnamon extract (10). The Olive leaf has antimicrobial and antioxidant (11), anti-inflammatory (12), gastro protective, anti-diabetes, anti-cancer, neuroprotective, hepatoprotective, cardioprotective, anti-obesity features (13, 14). Olive leaf has also medicinal benefits in type 2 diabetes. The aim of this study was to evaluate the protective effects of the organic extract of olive leaf on type 2 diabetes, and assessment of expression of liver superoxide dismutase and total antioxidant capacity of plasma in rats.

2. Material and methods

In a cross-sectional study, protective effects of the organic extract of olive leaf extract (*Olea europaea* L.) were assessed on sugar levels and expression of liver superoxide dismutase and total antioxidant capacity of plasma in rats with type 2 diabetes.

2.1. Extraction

After collecting the olive leaves from gardens around Shiraz, it was dried and were extracted. Extraction was performed by maceration technique. Briefly, 800 grams of dried leaf powder mixed with 3200 ml ethanol solvent for 24 under the hood. After 24, the solution was filtered by the filter paper and then, it was concentrated by rotary evaporator and weighted. Finally the extract was dissolved in distilled water.

2.2. Animal

In this study, 28 healthy, mature, Sprague-Dawely male rats with the initial weight of 250±50 g were divided into 4 groups, and were kept in separate cages (7 rats in one cage) and in an environmental condition with a temperature of 20-28 °C and humidity of 25-30%. During the study period, the rats freely were feed with standard diet (pellet) and water.

2.3. Inducing Type 2 Diabetes

The body weight of the rats were recorded, and in the fourth week, diabetes was induced in rats by intraperitoneal injection of 90 mg/ kg nicotinamide and 15 minutes after the injection of nicotinamide, a single dose of 50 mg/kg Streptozotocin in groups 3 and 4. In order to eliminate confounding factor (oxidative stress due injection) in control groups distilled water was injected. Seven days after inducing diabetes, fasting blood sugar was measured by using Pars Azmoon kit and GOD-PAP enzymatic method. The rats with blood sugar levels more than 250 mg/100 ml were considered as diabetic rats. To control rats, fasting blood sugar was measured once a week after 7-8 hours of starvation.

2.4. Treatment Modalities

Group 1: Healthy control group that received only standard diet (pellet) and water.

Group 2: Healthy control group that received olive leaf extract (100 mg/kg oral) during 8 weeks.

Group 3: Diabetic group that received normal diet for 8 weeks and then the 4th week one dose of 90 mg/kg nicotinamide were injected plus 50 mg/kg Streptozotocin, 15 minutes later.

Group 4: Diabetic group received 100 mg/kg olive leaf extract in addition to normal diet during 8 weeks, and in the 4th week, 90 mg/kg nicotine amide plus 50 mg/kg Streptozotocin, after 15 minutes.

2.5. Oral administration of Olive leaf extract

The rats' neck skin was taken by two fin-

Table 1. Primer sequences for real-time assessment of the expression of super oxide dismutase enzyme (SOD) and β-Actin.

Primer name		Primer sequences
SOD	Forward	TCCTTGCTTTTTGCTCTCCC
	Reverse	TGCTCGCCTTCAGTTAATCC
β-Actin	Forward	AGGACATCATCGGCAAT
		TGTGTTGGCATABAGGTCTT

gers and the gavage tube was sent to the esophagus parallel to throat of the rat. Administered dosage for each group was 100 mg/kg.

2.6. Surgery

At the end of the study, all rats were starved for 12, and were euthanized using ketamine and xylazine injected. After full anesthesia, blood sample was taken from their heart in tubes containing sodium citrate. Plasma generated by centrifuge at 3000 rpm for 10 min, and then was pout in microtubes, and were kept at -70 °C for measurement of biochemical markers.

2.7. Assessment of gene expression of super oxide dismutase enzyme (SOD)

Liver samples of the target groups were homogenized. RNA isolation was performed by RNX-Plus kit.

Real-time-PCR were performed by One Step SYBR® PrimeScriptTM RT-PCR Kit. After completing the reaction, and after plotting the threshold line, the values (quantity) of Ct and the gene expression was calculated by the $2^{-[(CT_{target_{test}}-CT_{actin_{test}})-(CT_{target_{control}}-CT_{actin_{control}})]}$ formula. β-Actin was used as a house keeping gene (internal gene).

2.8. Total antioxidant capacity of plasma (FRAP)

The total antioxidant capacity of the blood plasma was assessed by method of Benzie and colleague (15). In this method, the ability of plasma in regard to reduction of ferric ions was measured. Ferrous sulfate was used as standard in concentrations of 250, 500, and 1000 (µmol/lit).

2.9. Measurement of nitric oxide

Nitric oxide was assessed by the Griess reaction (16). For deproteiniziation with zinc sulfate, 400 µl of the sample was mixed with 6 mg of zinc sulfate powder. For measurement of nitrite and total nitrate concentration, 100 µl plasma was deproteinized, and then 100 µl vanadium chloride solution was added. After that, 100µl of sulfonamide/NED compound (1:1) was added to the plate and was incubated at 37°C for 30 min. After the reaction and color formation, light absorbance was measured in 540 nm wavelength and samples' concentration were determined by the standard curve. Sodium nitrite was used as the standard in concentrations of 3.125, 6.25, 12.5, and 25 (µmol/lit).

3. Results

The mean amounts of blood glucose in the 8th week for control healthy group (group1) was 85.14±10.65, for healthy rats received extract (group 2) was 87.86±7.56, for diabetic rats which did not receive extract (group 3) was 474.0±58 and for diabetic rats, which received extract (group 4)

Table 2. The results of the effect of extract of olive leaf on blood glucose levels in the studied groups (healthy and diabetic rats) after 8 weeks of administration of the extract.

Groups	FBS (mmol/lit)
Control	85.14±10.65
Control+Extract	87.86±7.56
Diabetic Rats	474.0±58.0
Diabetic Rats+Extract	439.4±46.8

Table 3. The effect of the extract of olive leaf on total antioxidant activity of the plasma after 8 weeks of administration of the extract.

Groups	TAC (µmol/lit)
Control	422.6±91.0
Control+Extract	379.9±56.8
Diabetic Rats	516.1±154.4
Diabetic Rats+Extract	435.5±38.7

was 439.4±46.8. At the end of the 8th week, the mean amounts of blood glucose was not significantly different between groups 1 and 2 (p=1), groups 3 and 4 (p=1), while there was significantly different between groups 2 and 3 (p=0.005), groups 1 and 3 (p=0.002), groups 2 and 4 (p=0.038), and groups 1 and 4 (p=0.015)(Table2).

The mean levels of the total antioxidant capacity of plasma are revealed in Table 3. The amounts of total antioxidant capacity of plasma were 422.6±91.0 in group 1, 379.9±56.8 in group 2, 516.1±154.4 for the group 3, and 435.5±38.7 for the group 4. There was no significant difference between total antioxidant capacity of plasma between groups 1 and 2 (p=0.99), groups 3 and 4 (p=0.99), groups 2 and 3 (p=0.35), and groups 1 and 3 (p=0.99), groups 2 and 4(0.86), groups 1 and 4(0.99) (Table 3).

Table 4. The results of the effect of organic extract of olive leaf on the amount of nitric oxide (NO) metabolites in the groups (healthy and diabetic rats) after 8 weeks of extraction.

Groups	NO (µmol/lit)
Control	8.92±4.22
Control+Extract	6.677±2.485
Diabetic Rats	3.764±1.686
Diabetic Rats+Extract	5.97±7.27

The mean levels of nitric oxide in groups 1 was 8.92±4.22, in the group 2 was 6.677±2.485, in the group 3, was 3.764±1.686, and for group 4 was 5.97±7.27. (Table 3) There was no significant difference for nitric oxide metabolite between groups 1 and 2 (p=0.99), groups 3 and 4 (p=0.99), groups 2 and 3 (p=0.28), and groups 1 and 3 (p=0.06), groups 2 and 4(p=0.509), groups 1 and 4 (p=0.126) (Table 4).

The mean levels of super oxide dismutase gene expression in the livers of the rats in groups 1, 2, 3 and 4 were 0.8700±0.1463, 0.04775±0.00885, 0.13250±0.00500, respectively. (Table 4). There was significant difference for the expression of superoxide dismutase enzyme gene in the rats of group 2 and 3 (p=0.005), whereas no significant difference was observed between groups 2 and 4 (p=0.338), and groups 3 and 4 (p=0.338) (Table 5).

Table 5. The results of the effect of the organic extract of olive leaf on the expression of the superoxide dismutase gene expression in the studied groups (healthy and diabetic rats) after 8 weeks of administration of the extract.

Groups	SOD
Control+Extract	0.8700±0.1463
Diabetic Rats	0.04775±0.00885
Diabetic Rats+Extract	0.13250±0.00500

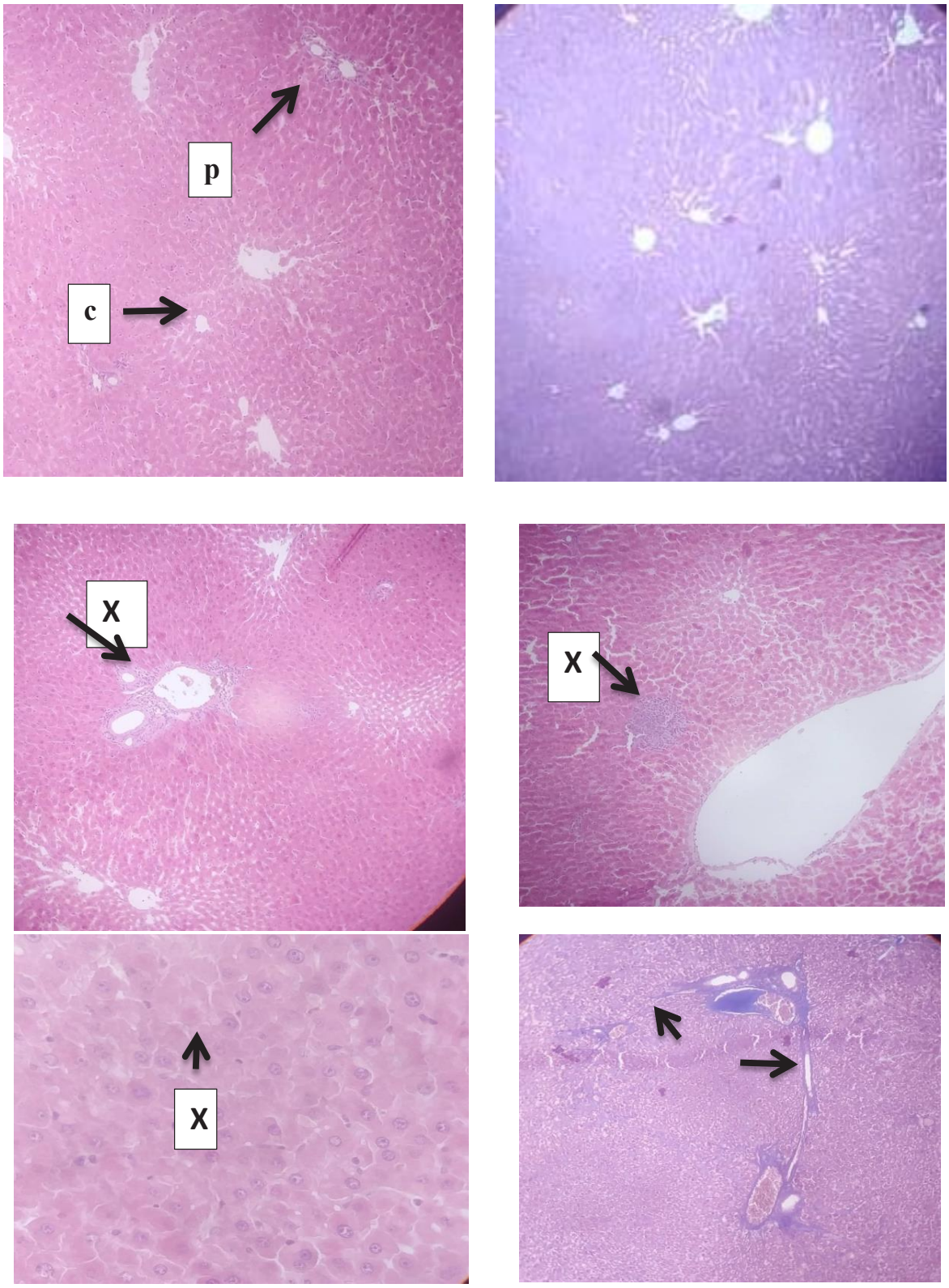

Figure 1. Histopathological changes of the liver of control and diabetic rats 8 weeks after administration of the extract.

3.1. Histopathological changes of the liver

NoNo histopathological changes were observed in the liver of healthy rats. According to the pathologist's opinion, histologically, no difference was found between the liver of healthy rats and the liver of healthy rat that received olive leaf extract. In the liver of diabetic rats that received olive leaf extract, mild portal chronic inflammation, accumulation of macrophages and a small amount of apoptosis and bridging fibrosis were observed (Figure 1).

4. Discussion

The aim of this study was to investigate the effect of the alcoholic extract of olive leaf on the prevention of type 2 diabetes, liver superoxide dismutase enzyme expression and total antioxidant activity of plasma in streptozotocin-induced diabetic rats (17). The extract of the olive leaf has been reported to have anti-hyperglycemic (18, 19), antioxidant (11, 20), blood pressure lowering (21, 22), anti-cancer (23) and anti-inflammatory (24) and antimicrobial properties (25).

In this study, there was no significant changes between the group 3 and group 4 as well as the group 1 and group 2. In one study conducted by Julio Wainstein et al. (18), olive leaf extract showed significant effects on glucose homeostasis, reducing starch digestibility and absorption, and controlled glucose homeostasis. Also, the aqueous extract of olive leaves had a significant effect on reducing blood glucose in rats (26). In other study, it was found that olive leaf polyphenols had recovery effects on insulin resistance leading to improving glucose hemostasis in diabetic individuals (27). In a human clinical trial study conducted by Wainstein and colleagues, olive leaf extract was effective for the treatment of diabetes (18). In addition to the differences existing in the design of the present animal study and the human study, the controversial results of glucose levels and the ineffectiveness of the olive leaf extract on the reduction of blood glucose in the diabetic group may have different reasons, for example, low dosage of the extract in the present animal study compared to the dosage used in the human study, or it may be due to the short duration of the intervention of the present study.

In this study, no significant difference was observed for the total antioxidant activity between the control and experimental groups in fasting state. In one study conducted by Jemai et al, the anti-diabetic properties of hydroxytyrosol and oleuropein were investigated in alloxan induced diabetic rats, and they found that the hydroxytyrosol and oleuropein significantly reduced blood glucose levels and improved total antioxidant activity in rats (28). In this study, olive leaf extract did not show a significant effect on total antioxidant activity.

In another study, FayadhAl-Azzawie et al. (29) evaluated the anti-diabetic and antioxidant properties of oleuropein as one of the active ingredients of olive leaf for 16 weeks in rabbits, and found that this agent had a significant effect on the reduction of blood glucose level and antioxidant activity (MDA oxidative stress marker levels).

The effect of organic extract of the olive leaf on the levels of nitric oxide metabolites in healthy and diabetic rats was also evaluated. It was found that the levels of nitric oxide metabolites did not have any significant difference between control and diabetic rats. In one study, nitric oxide levels changed depending on the dosage of oleuropein (30). The effect of organic extract of olive leaf on the expression of superoxide dismutase gene in healthy rats showed a significant difference with the diabetic control group, while no significant difference was found between the diabetic control rats and the diabetic rats receiving extract, also between the healthy control group and the diabetic group receiving the extract. In one study, the olive leaf extract showed a strong neuroprotective effect on neuronal damage in the hippocampus after cerebral ischemia, which can be attributed to its antioxidant properties (31).

5. Conclusion

According to the results of this study, it can be concluded that the alcoholic extract of olive leaf with a dosage of 100 mg/kg did not reduce blood glucose in diabetic rats. Therefore, traditional methods used to treat diabetes type 1 and 2 do not only prevent but also diminish the ability to reduce blood sugar and can also cause damage to hepatocytes and other complications.

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Conflict of Interest

None declared.

6. References

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