Effect of Ethanolic Olive leaf and its Callus Ethanol Extracts in Alloxan-Induced Diabetic mice (Blood glucose and lipid profiles)

تأثيرات مستخلصات ورق الزيتون والكالس على بعض المعايير الكيمياء الحيوية والمناعية في الفئران المصابة بالسكري المحدث تجريبيا بواسطة الالوكسان

Sally Badawi **Saleh Ahmed** **Nabeel Al -Ani**
College of Science/ Al- Nahrain University

سالي بدوي شبيب **صالح احمد وهيب** **نبيل خلف العاني**
كلية العلوم / جامعة النهرين

Abstract:
This study was designed to test the lipid-lowering and antidiabetic activities of olive leaf and its callus extract. Diabetes in mice was induced by intraperitoneal injections of alloxan. The serum glucose and serum lipid were examined. Diabetic mice showed hypeglycemia, hypelipidemia. The administration, for 2 weeks of olive leaf and its callus extracts significantly decreased the Total cholesterol (TC). Triglycerides (TG). Low density lipoprotein (LDL), very low density lipoprotein (VLDL). Both types of olive extracts had significant hypoglycemic effects on blood glucose levels in diabetic mice. This hypoglycemic effect was as potent as the hypoglycemic effect of insulin. However, the callus extract was more potent than the leaves extracts and most potent than insulin in causing a significant decrease in LDL, VLDL, TC, TG and in antidiabetic effects.

المستخلص
يهدف البحث لدراسة تأثير مستخلص اوراق وكالس الزيتون على انخفاض مستوى الدهون ومستوىالسكر في الدم . تم تحفيز السكري في الفئران بواسطة الحقن الوريدي من الالوكسان وتم قياس السكر والدهون . لوحظ انخفاض بمستوى السكر والدهون عند الفئران المصابة بالسكري ، تم اعطاء المستخلص لمدة اسبوعين للفئران المصابة بالسكر ووجد ان له تاثير واضح في خفض مستوى الكولسترول والكلسيريدات الثلاثية والدهون واطئة الكثافة والدهون ذات الكثافة الواطئة جدا ، كلا النوعين من المستخلص كان لهما تأثير واضح في خفض مستوى السكر في الدم عند الفئران المصابة بالسكري ، اما بالنسبة لانخفاض السكر كان للكالس نفس تأثير الانسولين بينما كان له تأثير اكبر في خفض مستوى الكولسترول والكلسيريدات الثلاثية والدهون واطئة الكثافة والدهون ذات الكثافة الواطئة جدا.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide that caused by an absolute or relative lack of insulin and or reduced in insulin activity [1]. It is characterized by hyperglycemia and long-term complications affecting the eyes, kidneys, nerves and blood vessels. Although the leading mechanism of diabetic complications remains unclear. Much attention has been suggested that oxidative stress may contribute to the pathogenesis of different diabetic complications [2].

Key words: Diabetic, Olive, leaves, callus, Alloxan

peroxidation, alteration of the glutathione redox state, a decrease in the content of individual natural antioxidants and finally reduction in the antioxidant enzyme activities. These changes suggest an oxidative stress caused by hyperglycemia [3, 4].

Nowadays herbal drugs are gaining popularity in the treatment of diabetes and its complications. One of these hypoglycemic plants is *Olea europaea* which belongs to Oleaceae family. The olive tree is one of the most important trees in Mediterranean countries. It grows through the European countries [5]. The fruits oil are important components in the daily diet of a large part of the world's population, as the leaves was important for their contents of secondary metabolites. They consist of phenolic compounds, flavonoids and volatiles oil [6]. Wojicikowski studied the antioxidant capacity of 55 medicinal plants, and found olive leaf extract had the highest radical scavenging activity [7]. Therefore, it has been proposed that inhibition of the generation of the oxidative LDL-generated foam cells and reductions in the level of TG, TC, LDL, VLDL by naturally occurring compounds. Phenolic compounds from various sources have been reported to prevent LDL oxidation *in vitro* and show marked hypolipidemic activity *in vivo* [8, 9].

Materials and methods

Plant material

The leaves of *Olea europaea* from newly branches of apical meristem which is found in garden, we divide these leaves into two parts:

Part I: the leaves were dried and kept at room temperature until use.

Part II: leaves were sterilized and cultured on the nutrient medium for the preparation of the callus.

Sterilization of leaves

Leaves are rinsed with tap water for 10 min, then transferred to laminar air flow cabinet where submerged in sodium hypochlorite at 0.5% for 2 min. They were rinsed three times in sterilized distilled water, for 5 minutes.

Medium and culture conditions

The leaves were placed on Murashige and Skooge media supplemented with 30g/L [10]. The medium was supplemented with 1.5 mg/L Benzyl adenine and 0.5mg/L 2,4-Diclorophenoxyaceticacid. The pH of medium adjusted to 5.7 by HCl or NaoH prior to adding 7g/L and the autoclaved at 121C° and 1.04 Kg/cm² for 15 min [11]

Olive leaf extracts preparation

The dried leaves were powdered using a coffee grinder and then extraction. 50 g of the processed plant were extracted in 250 of ethanol (70%) using the soxhlet apparatus. The obtained extract was then evaporated at 37C° in the incubator and the resultant crude extract was frozen at -20C° until use [12].

Olive callus extract preparation

A quantity of (5g) of callus powder was mixed with 25 ml of 70 % ethanol then placed in soxhlet apparatus for 6 hrs. at (40-60C)°, and then the solvent was removed under reduced pressure by rotary evaporator at 40 C° [13]

Animals and treatments

Female mice obtained from the national center for drug control and research. The animals were kept at controled environment, in breeding room (temperature 20C°; 12h dark/light cycle). All mice had access to a standard laboratory diet and were fasted overnight before blood collection. Diabetes was induced in mice by a single intraperitoneal injection of freshly prepared alloxan solution in normal saline at a dose of 100 mg/kg body weight [14]. The feeding experiment was carried out for a period of 2 weeks after the induction of diabetes in 5 days (characterized by the presence of glucosuria). The mice were divided into 7 groups consisting of 5 mice each. Group I (normal control) consisted of normal mice. Group II (untreated diabetic mice), Group III diabetic mice treated with insulin, Group IV (diabetic mice treated with olive leaves ethanolic extract at a dose 500 mg/kg, Group V (diabetic mice treated with olive leaves ethanolic extract at a dose 250 mg/kg), group VI (diabetic mice treated with olive callus ethanolic extract at a dose 500 mg/kg), group VII (diabetic mice treated with olive callus ethanolic extract at a dose 250 mg/kg).

Serum glucose

Concentration of glucose in serum was measured using commercial kits from Linear (linear, Italy).

Serum lipid

Concentrations of total cholesterol (TC), triglycerides (TG), LDL, HDL, and VLDL in serum were determined by enzymatic colorimetric methods using commercial kits from Linear (Linear, Italy).

Results

Chemical detection of plant extract

Chemical detection of *Olea europaea* ethanol extract revealed that flavonoids, phenols, tannins, saponins, terpenes, glycosides, steroids were detected in extract and negative for alkaloid.

Blood glucose

The blood glucose level in distilled water treated mice (control) was observed during the period of treatment, which revealed that experimental conditions (nutrition, humidity, light) did not influence the blood glucose level. Untreated diabetic mice showed a significant increase in blood glucose levels as compared with control. Insulin treatment significantly decreased glucose level in diabetic mice. Both type of olive leaves and callus extracts had significant hypoglycemic effects on blood glucose levels in diabetic mice. This hypoglycemic effect was as potent as the hypoglycemic effect of insulin Table (1)

Table (1): Blood glucose level of alloxan –induced diabetic mice after treatment with insulin and ethanolic leaf and callus extracts of *O.europaea*

Treatment Groups		Dose (mg/kg Body wt.)	Blood glucose (mg/dL)
control		0.0	100.23 ±5.52 C
Untreated diabetic mice		0.0	519.04 ± 8.71 A
Insulin		10 U	205.29± 5.75 B
Treated Diabetic Mice	Ethanolic (olive leaf)	250	221.79 ± 2.93 B
	Ethanolic (olive leaf)	500	219.61± 1.68 B
	Ethanolic (olive callus)	250	208.68 5.53 B
	Ethanolic (olive callus)	500	204.67 ±9.15 B

means having different letters are significant different ($P \leq 0.05$)
Values are expressed as Mean ± SE

Lipid profile

Untreated diabetic mice showed a significant increase in TC, TG, LDL, VLDL level as compared with control. Insulin treatment significantly decreased TC, TG, LDL, and VLDL in these animals. Both doses of ethanolic leaf extract showed a significant decrease in TC level compared to non treated diabetic group, but cholesterol level remained significantly higher than that in the insulin treated group. Triglycerides level significantly decreased to values comparable to those induced by insulin. On the other hand, the effect of ethanolic leaf extracts were equipotent to the effect of insulin in lowering VLDL level in treated diabetic mice, managed to normalize LDL level and comparable to the effect of insulin on HDL level. Both doses of ethanolic callus extract showed an equipotent and more decrease in TC, TG, LDL, VLDL, compared to insulin and treated diabetic groups, and normalized cholesterol levels. However, the callus extract was more potent than the leaves extracts. Table (2, 3, 4, 5, 6).

Table (2): Cholesterol level of alloxan –induced diabetic mice after treatment with insulin and ethanolic leaf and callus extracts of *O.europaea*

Treatment Groups		Dose (mg/kg Body wt.)	Cholesterol (mg/dL)
control		0.0	79.2 ± 3.54 D
Untreated diabetic mice		0.0	116 ± 7.03 A
Insulin		10 U	88 ± 4.91 C
Treated Diabetic Mice	Ethanolic (olive leaf)	250	102 ± 4.83 B
	Ethanolic (olive leaf)	500	100 ± 4.70 B
	Ethanolic (olive callus)	250	77 ±3.91 D
	Ethanolic (olive callus)	500	74 ±3.86 D

($P \leq 0.05$) means having different letters are significant different
Values are expressed as Mean ± SE

Table (3): Triglycerides level of alloxan –induced diabetic mice after treatment with insulin and ethanolic leaf and callus extracts of *O.europaea*

Treatment Groups		Dose (mg/kg Body wt.)	Triglycerides (mg/dL)
control		0.0	47.6 ± 2.77 D
Untreated diabetic mice		0.0	181 ± 9.74 A
Insulin		10 U	75 ± 3.41 C
Treated Diabetic Mice	Ethanolic (olive leaf)	250	96 ± 4.57 C
	Ethanolic (olive leaf)	500	94 ± 3.98 C
	Ethanolic (olive callus)	250	53 ±2.64 D
	Ethanolic (olive callus)	500	51 ±2.51 D

(P ≤ 0.05) aving different letters are significant different
Values are expressed as Mean ± SE

Table (4): VLDL level of alloxan –induced diabetic mice after treatment with insulin and ethanolic leaf and callus extracts of *O.europaea*

Treatment Groups		Dose (mg/kg Body wt.)	VLDL (mg/dL)
control		0.0	9.5 ± 0.79 E
Untreated diabetic mice		0.0	37 ± 2.17 A
Insulin		10 U	15 ± 0.84 D
Treated Diabetic Mice	Ethanolic (olive leaf)	250	19 ± 1.32 CD
	Ethanolic (olive leaf)	500	19 ± 1.32 CD
	Ethanolic (olive callus)	250	11 ±0.72 E
	Ethanolic (olive callus)	500	10 ±0.63 E

(P ≤ 0.05) means having different letters are significant different
Values are expressed as Mean ± SE

Table (5): HDL level of alloxan –induced diabetic mice after treatment with insulin and ethanolic leaf and callus extracts of *O.europaea*

Treatment Groups		Dose (mg/kg Body wt.)	HDL (mg/dL)
control		0.0	33.2 ± 2.36 BC
Untreated diabetic mice		0.0	25 ± 1.53 C
Insulin		10 U	39 ± 1.97 B
Treated Diabetic Mice	Ethanolic (olive leaf)	250	36 ± 2.78 B
	Ethanolic (olive leaf)	500	37 ± 2.84 B
	Ethanolic (olive callus)	250	46 ±2.65 A
	Ethanolic (olive callus)	500	50 ±2.86 A

(P ≤ 0.05) means having different letters are significant different
Values are expressed as Mean ± SE

Table (6): LDL level of alloxan –induced diabetic mice after treatment with insulin and ethanolic leaf and callus extracts of *O.europaea*

Treatment Groups		Dose (mg/kg Body wt.)	LDL (mg/dL)
control		0.0	35 ± 2.53 BC
Untreated diabetic mice		0.0	55 ± 2.87 A
Treated Diabetic Mice	Insulin	10 U	34 ± 1.66 C
	Ethanolic (olive leaf)	250	46 ± 2.74AB
	Ethanolic (olive leaf)	500	42 ± 2.65 B
	Ethanolic (olive callus)	250	20 ±1.16 D
	Ethanolic (olive callus)	500	13 ±0.81 D

($P \leq 0.05$) means having different letters are significant different
Values are expressed as Mean ± SE

Discussion

Recently, much attention has been focused on antioxidants in food that are potential compounds for preventing diseases caused by oxidative including diabetes because of their distinctive biological activity and low toxicity. This result is agrees with [15] who reported the hypoglycemic action of *O.europaea* extract Similar finding was reported by [16] who found that oral administration of the olive leaves extract at (100,250,500) mg/kg body weight, for 14 days significantly decreased the blood glucose level in diabetic rats.

[17] suggested that hypoglycemic effects of plants might be attributed to the presence of insulin – like substances, stimulation of B- cell to produce more insulin, and a high level of fiber which interferes with carbohydrate absorption or the regenerative effect on pancreatic tissue.

The diabetogenic effect of alloxan was attributed to its ability to destruct pancreatic β islets cells, possibly by free radical mechanism. Diabetes, therefore, represents a state of increased lipid peroxidation and reduced antioxidant reserve [18].

It has been demonstrated that hyperglycemia of diabetes can generate oxidative stress, manifested by the presence of free radicals with the simultaneous decline of antioxidant defense mechanisms observed in diabetic patients, a finding that could promote the development of diabetic complications [19]. Furthermore, hypercholesterolemia, especially elevated plasma LDL, and hypertriglyceridemia are independent risk factors that alone or together can accelerate the progression of atherosclerotic lesions [20].

In conclusion, this study demonstrated that, the callus extract was more potent than the leaves extracts. The reason of these results may belongs to the used growth regulators which stimulate and maintain the callus, where these regulators stimulate and increase the production of secondary metabolite in callus, sub culturing may also have a role in somaclonal variation initiation in cells which leads to increasing its productivity of secondary metabolite.

The stages of callus induction, maintenance and the optimum conditions for cultures are particular stimulators led to increasing in production of secondary metabolites in callus of newly synthesized leaves as compared with the mother plant [21].

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