

Effect of aqueous extract of olive (*Olea europaea*) fruit on lipid profile in female rabbits

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Abstract:

The activity of the aqueous extract of *Olea europaea* was tested at concentrations of 8, 15 or 20 mg/kg of body weight on lipid profile in twenty female local rabbits. These animals were randomly divided into four groups (five animals in each group). Three groups were dosed orally with the concentrations mentioned above, while the last was administered with distilled water and considered as a control group. These animals were orally dosed by aqueous extract using a micropipette for 30 days.

The results showed that there was a significant ($P < 0.05$) decrease in cholesterol, triglycerides, low density lipoprotein (LDL-cholesterol), very low density lipoprotein (VLDL-cholesterol) concentrations and atherosclerosis index means for the three treated groups with the aqueous extract of olive fruit compared with the control group. The results also showed that there was a significant ($P < 0.05$) increase in high density lipoprotein (HDL-cholesterol) for the three treated groups as compared with the control group.

In conclusion, the diet rich in olive fruit extract may decrease the risk of coronary heart disease by inhibiting LDL oxidation and improving the lipid profile.

Key words: olive, *Olea europaea*, cholesterol, triglycerides, high density lipoprotein, low density lipoprotein.

Introduction:

The olive trees (*Olea europaea*), which constitute a regular dietary component and a source of compounds that have important biological properties, grow all over the world except for cold areas and the Arctic. They grow mainly in temperate and equatorial regions such as south west Asia [1]. The olive is native to the mediterranean region, tropical and central Asia and various parts of Africa [2].

Olive oil is made up of important chemical compounds, including glycerol compounds and small quantities of free fatty acids. The

combination of glycerol and fatty acids known as glycerides [2]. It also contains pigments, aroma compounds, tocopherols, squalene (up to 0.7%), sterols (about 0.2% phytosterol and tocosterols), unidentified resinous components and others phenols and a group of related natural products with potent antioxidant properties [3, 4].

In the modern medicine, it is well accepted that the high monounsaturations of olive oil and the presence of several other constituents such as phenols (mainly oleuropein and hydroxytyrosol), tocopherols and other compounds exhibit a significant

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role on the health [3]. Therefore, the diet rich in olive has a beneficial effect on diseases associated with oxidative damage such as coronary heart disease (CHD) and cancer, and also on aging. Olive oil consumption was associated with a lower coronary risk and with a reduced breast-cancer risk [5]. Animal experiments in rabbit and rat preparations found a hypotensive effect of oleuropein, possibly via direct action on smooth muscle. Oleuropeoside also may exert vasodilator activity. Olive extract may posse antispasmodic, vasodilator and anti-arrhythmic properties [6].

Studies in laboratory animals have reported the hypoglycemic and hypolipidemic activity of olive leaf and the active constituent was oleuropein which showed this activity [6, 16]. Therefore, the aim of this work was to study the effect of the aqueous extract of olive fruit on lipid profile in female rabbits.

Materials and methods:

Preparation of extract

Olea europaea fruits were washed, cut into small pieces, dried in oven at 40° C (the olive pieces were dried at this temperature in order to conserve the active materials in olive), and ground. The powder was mixed with five folds of distilled water. Then, the mixture was put in the reflux at 100° C temperature for three hours. After that, the extract was filtered and put in the rotary evaporator to concentrate the fluid. Then, the crude extract was further dried in oven at 45° C [7]. After drying, the crude extract was collected and stored at -20° C until used [8].

Experimental animals

Twenty female local rabbits with an average age of about 3-3.5 months and weight between 1150 – 1600 g were used. They were bred in

special cages in Al- Nahrain University Research Center for Biotechnology, fed pellets (contain 20 % crude protein and 11% crude fibre, rich in protein and energy) and given tap water *ad libitum* during the experimental period which last from February to April. Concerning conditions of the laboratory, average temperature was about 21 - 24° C. and the light cycle was divided into 12 hours light: 12 hours dark [9].

Doses and design of the experiment

Female rabbits were dosed orally by aqueous extract using a micropipette. The powder was mixed with distilled water to prepare the different doses of extract. The volume of administrated dose was 1ml/ day for 30 days. These doses were determined through the amount of effective dose for human. The effective dose was 400 mg/ kg for olive extract [17]. Therefore, it was selected the concentrations 8, 15 and 20 mg/kg of body weight.

The animals were randomly divided into four groups (five animals in each group). The first, second and third groups were dosed with 8, 15 and 20 mg/kg of body weight, respectively, while the last group was considered as control and daily administrated with 1 ml distilled water.

Blood sample collection

After the period of dosing was elapsed (30 days), blood was collected by heart puncture. The volume of blood was 8 ml and collected in glass tubes. The blood sample slowly expressed into the vial to reduce the risk of hemolysis after removing of the needles from syringes [10].

Serum was separated by putting the tubes in the centrifuge at 3000 rpm for 15 min at 37°C. After the collection of serum by Pasteur's pipettes, serum

samples were stored at -4°C until biochemical tests were performed [5].

Biochemical parameters

Total cholesterol, HDL cholesterol and triacylglycerides were determined using assay kits (Biomaghreb Company, Tunis) for *in vitro* diagnosis use [5, 11]. The kits for total cholesterol and triglycerides determination depend on enzymatic hydrolysis, while the kit for HDL cholesterol determination depend on the precipitation reaction and supernatant formation.

Concerning the LDL-cholesterol concentration, Friedewald equation was used as below:

$$[\text{LDL- chol}] = [\text{Total- chol}] - [\text{HDL- chol}] - [\text{VLDL- chol}]$$

The VLDL- chol concentration was calculated as below:

$$[\text{VLDL- chol}] = \text{TG}/5, \text{ while the atherosclerosis index was calculated by dividing the LDL- cholesterol on the HDL- cholesterol [12].}$$

Statistical analysis

The results were analyzed statistically using analysis of variance (ANOVA) applicable to a completely randomized design. Then, the significance among means was tested depending on Duncan Multiple Range Test using SPSS program [13, 14].

Results and discussion:

Table (1) illustrates the effect of aqueous extract of olive fruit on means of cholesterol and triglycerides concentrations in female rabbits. The results showed that there was a significant ($P < 0.05$) decrease in cholesterol concentration in the three groups treated with aqueous extract of olive compared with control animals. The cholesterol concentration mean was 107.074 mg/dl in control group, while it was 94.500, 91.922 and 90.867 mg/dl in treated groups with 8, 15 and

20 mg/kg of body weight, respectively. The results also showed that there was a significant ($P < 0.05$) decrease in triglycerides concentration in animals treated with the three concentrations of the aqueous olive fruit extract compared with control group. The means were 116.877, 98.459, 96.699 and 95.136 mg/dl for control, 8, 15 and 20 mg/kg of body weight, respectively.

Table (1): Effect of aqueous extract of olive fruit on cholesterol and triglycerides concentrations in female rabbits (Mean \pm SE).

Treated groups	Cholesterol concentration (mg/dl)	Triglycerides concentration (mg/dl)
8 mg/kg	^b 94.500 \pm 1.521	^b 98.459 \pm 1.607
15 mg/kg	^b 91.922 \pm 1.553	^b 96.699 \pm 1.203
20 mg/kg	^b 90.867 \pm 1.505	^b 95.136 \pm 1.531
Control	^a 107.074 \pm 2.133	^a 116.877 \pm 1.377

* Similar letters indicated that there were no significant differences between treatment groups and different letters indicated that there were significant differences between treated groups at $p < 0.05$.

Table (2) shows the effect of aqueous extract of olive on HDL, LDL, VLDL-cholesterol concentrations and atherosclerosis index in female rabbits. The results revealed that there was a significant ($P < 0.05$) increase in HDL-cholesterol concentration for the three groups treated with aqueous extract of olive fruit compared with the control animals. The HDL-C means were 46.861, 52.872 and 55.775 mg/dl in animals treated with 8, 15 and 20 mg/kg of body weight, respectively, while the mean in the control group was 42.445 mg/dl in control group. The results also demonstrated that there was a significant ($P < 0.05$) decrease in LDL and VLDL-cholesterol concentration for the three groups treated with aqueous extract of olive fruit compared with the control. The LDL-C means were 41.253,

27.947, 19.710 and 16.064 mg/dl for control, 8, 15 and 20 mg/kg, respectively, while the VLDL-C means were 23.375, 19.691, 19.339 and 19.027 mg/dl for control, 8, 15, 20 mg/kg of body weight, respectively.

Concerning the atherosclerosis index, the results showed that there was a significant ($P < 0.05$) decrease for the three groups treated with aqueous extract of olive fruit compared with the control. The means were 0.980, 0.597, 0.372 and 0.287 mg/dl for control, 8, 15 and 20 mg/kg of body weight, respectively.

Table (2): Effect of aqueous extract of olive fruit on HDL, LDL and VLDL-cholesterol concentrations and atherosclerosis index in female rabbits (Mean \pm SE).

Treatment groups	HDL-cholesterol	LDL-cholesterol	VLDL-cholesterol	Atherosclerosis index
8 mg/kg	^b 46.861 \pm 0.963	^b 27.947 \pm 1.142	^b 19.691 \pm 0.321	^b 0.597 \pm 0.032
15 mg/kg	^a 52.872 \pm 0.664	^c 19.710 \pm 1.375	^b 19.339 \pm 0.240	^c 0.372 \pm 0.026
20 mg/kg	^a 55.775 \pm 0.764	^c 16.064 \pm 1.011	^b 19.027 \pm 0.306	^c 0.287 \pm 0.015
Control	^c 42.445 \pm 1.650	^a 41.253 \pm 2.213	^a 23.375 \pm 0.275	^a 0.980 \pm 0.071

* Similar letters indicated that there were no significant differences between treatment groups and different letters indicated that there were significant differences between treated groups at $p < 0.05$.

The results of the present study came into agreement with the results of [15] in that higher serum high-density lipoprotein cholesterol, lower ratio of total cholesterol to high-density lipoprotein and lower triacylglycerols occurred after the olive oil diet was given to healthy individuals. The significant decrease in LDL-C concentrations in treated animals with the aqueous olive fruit extract was in accordance with the result of [3] in which diets rich in olive oil produced have a stable ratio between total cholesterol and HDL with a reduction of LDL-cholesterol in human. In addition, it has been found that dietary olive oil decreased susceptibility of the

lipoprotein to undergo lipid peroxidation, after 1 week of olive oil diet was given to men [4]. The decrease in the LDL-cholesterol, which is responsible for the formation of the atherosclerotic plaque, might be related to the presence of oleic acid in olive extract. This acid also acts to increase the HDL-cholesterol [3].

The hypocholesterolemic effect of this extract might be related to the presence of oleuropein, oleuropein aglycone and hydroxytyrosol in olive extract. These phenolic compounds had the abilities to lower serum TC, TG and LDL-C levels as well as slowing the process of lipid peroxidation and enhancing antioxidant enzyme activity, such as superoxide dismutase (SOD) and catalase [16]. In addition, it has been found that the phenolic and flavonoid compounds in olive oils may act to increase the HDL-C [5]. Furthermore, an increase in squalene level; a compound found in olive fruit, might lead to the inhibition of the enzyme that activate biosynthesis of cholesterol in the liver. This inhibition may cause a decreased in the production of cholesterol and the intermediates formed during its biosynthesis. Chronic long-term administration of squalene might also result in increased fecal elimination of cholesterol [17].

In conclusion, there is no doubt that diet rich in olive may decrease the risk of coronary heart disease through a decreased formation of atherosclerotic plaques by inhibiting LDL oxidation and improving the serum lipid profile.

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تأثير المستخلص المائي لثمرة الزيتون *Olea europaea* في صورة الدهون لإناث الأرانب

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الخلاصة:

اُختبرت فعالية ثلاثة تراكيز مختلفة (8 و 15 و 20 ملغم/كغم من وزن الجسم) من المستخلص المائي لثمرة الزيتون في صورة الدهون لعشرين انثى من الأرانب المحلية. قسمت هذه الحيوانات عشوائياً على أربع مجموعات بواقع خمسة حيوانات للمجموعة الواحدة. جرعت المجموع الثلاث الأولى فموياً بالتراكيز المذكورة سابقاً من المستخلص فيما جرعت المجموعة الرابعة بالماء المقطر بوصفها مجموعة سيطرة بالمستخلص المذكور فموياً باستخدام الماصة الدقيقة ولمدة 30 يوماً.

أظهرت النتائج أن هناك انخفاضاً معنوياً ($P < 0.05$) في معدل تركيز الكوليستيرول والشحوم الثلاثية والبروتين الدهني الواطئ الكثافة (LDL) والبروتين الدهني الواطئ الكثافة جداً (VLDL) ودليل تصلب الشرايين للمجموع الثلاث المعاملة بالمستخلص المائي لثمرة الزيتون مقارنة بمجموعة السيطرة. كما بينت النتائج أن هناك ارتفاعاً معنوياً ($P < 0.05$) في البروتين الدهني عالي الكثافة (HDL) للمجموع الثلاث المعاملة مقارنة بمجموعة السيطرة.

يمكن الاستنتاج من هذه الدراسة أن المستخلص المائي لثمرة الزيتون يمكن أن يقلل من أخطار أمراض الشرايين التاجية القلبية من خلال تثبيطه لأكسدة البروتين الدهني واطئ الكثافة (LDL) وتحسين صورة الدهون.