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Antidiabetic effect of extra virgin olive oil from the 'Rougette' variety of the Skikda region of eastern Algeria

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Abstract

The objective of this study is to determine the effects of an olive oil from the local "Rougette" variety on fasting glucose and lipid profile in rats with streptozotocin diabetes. For this, albino Wistar male rats were randomly divided into 4 groups. Rats were made diabetic by injection of streptozotocin. All groups received a standard rodent diet. Group 1: control received only this diet, both groups (2, 3): non-diabetic and diabetic received 2 ml of fasting extra virgin olive oil (EVOO) orally for 38 days, group 4: diabetic untreated. At the end of the experiment, body weights, fasting glucose, and lipid profile were measured. The results showed that there was an increase in body weight, a significant decrease in fasting blood glucose and triglycerides (TG), a highly significant increase in high density lipoprotein (HDL) and a decrease in total cholesterol (TC) in rats of the diabetic group treated with extra virgin olive oil (EVOO) compared with the untreated diabetic group. In control and healthy rats treated with olive oil, no significant difference was observed. In conclusion, olive oil can significantly improve fasting blood glucose and lipid profile in diabetic rats.

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Introduction

The olive tree (*Olea europaea* L.) is a tree that is found around the Mediterranean basin and belongs to the oleaceae family, characterized by its fruit, the olive and an oil from it. Olive oil is rich in monounsaturated fatty acids, mainly represented by oleic acid C18: 1 ω9, and in minor compounds such as tocopherols, phenolic compounds, sterols and aromatic compounds (Ghedira, 2008).

It is important to mention that "extra virgin" olive oil is the only oil with such virtues because it comes directly from the pressure of the fruit, without any treatment. It therefore retains the nutritional qualities of the olive and its components such as polyphenols (Doveri *et al.*, 2007).

Olive oil has essentially anti-oxidant, antihypertensive, platelet aggregation inhibitors that prevent cardiovascular disease (Ghedira, 2008).

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from a defect in the secretion of insulin or the action of insulin or these two associated abnormalities (Drouin *et al.*, 1999).

The treatment of diabetes is to target normal glycemic control as possible while avoiding hypoglycemia. Many natural antioxidants are used in the treatment of diabetes. In diabetic patients, they reduce the harmful effects of oxidative stress and free radicals (Lean *et al.*, 1999; Asgary *et al.*, 2002). Olive oil being rich in antioxidants could play the same effects.

Diets rich in monounsaturated fatty acids such as extra virgin olive oil, improve lipid profiles and glycemic control in diabetics, suggesting that high dietary intake of these diets improves insulin sensitivity. (Ros, 2003; Paniagua *et al.*, 2007).

In this study, we tried to verify if extra virgin olive oil from a local olive variety "Rougette" (ITAFV, 2002) has an effect on fasting blood glucose and lipid profile in non-diabetic and diabetic rats streptozotocin (STZ).

Materials and methods

Fatty acid composition

Transesterification

100 mg of olive oil was placed in a 5ml test tube with 2ml of heptane under stirring. Then 0.2ml of a methanolic solution of KOH 2N have been added, the test tube was strictly sealed and vigorously stirred for 30 sec. After stratification of layers the upper organic solution containing the methyl ester mixture was separated and directly used for gas chromatographic analysis.

GC and GC-MS Analysis

A Shimadzu GC-17A Gas Chromatograph equipped with a fused silica capillary column (DB5-MS30 m x 0.25 mm x 0.2µm) coupled with a flame ionization detector (FID). The operating conditions were the following: 100 °C for 1 minute, then until 280°C at 5°C/min and held at 280°C for 15 minute; injector temperature 250°C; detector temperature 280°C; carrier gas helium (1mL/min); split mode (1:92), volume of injection 1µL. Percentages of compounds were determined from their peak areas in the GC-FID profiles. GC-MS analyses were performed on a Shimadzu GCMS-QP5050A with the same column and the same operative conditions used for analytical GC. Ionization was performed at 70 eV. Ion source temperature 180°C; mass spectral data were acquired in the scan mode in *m/z* range 40-400. Oil solutions were injected with the split mode (1:96).

Animals and diet

Twenty-four albino Wistar weighing rats (140-150g) from the Pasteur Institute of Algiers were separated and divided into groups of 6 rats per cage with *ad libitum* access to water and a standard diet for rats (UAB: National Animal Feeding Unit, Bejaia).

They were maintained under conditions of temperature and light (24 ± 1°C and 12 h light / dark cycle) respectively and strict hygiene for a few days, for acclimation. The experiments were conducted according to the guidelines in the Guide to the Management and Use of Laboratory (Animals Committee for the Update of the Guide for the Care and Use of Laboratory Animals, National Research Council. Guide for the Care and Use of Laboratory Animals)

Induction of diabete

Diabete was induced after a fast night of rats by injection of streptozotocin at a dose of 60mg /kg body weight intravenously. Streptozotocin induces diabetes within three days by destroying Langerhans beta cells (Karunanayake *et al.*, 1975). Due to acute hypoglycemia, rats received 10% sucrose solution for 48h in place of water. Blood samples were collected from the terminal part of the tails. The blood glucose was measured by glucometer. Rats with a blood glucose level ≥ 300mg/dl (16.7 mmol/l) were considered diabetic. (Alirezaei *et al.*, 2012).

Experimental protocol

The experiment was started just after the onset of diabetes and the selection of six diabetic rats. The experience was 38 days (May 12, 2016-June 18, 2016).

The groups are distributed as follows: 1st group as a control (Control), 2nd group as untreated diabetic (DIAB), 3rd group: non-diabetic rats receives (02ml) extra virgin olive oil (EVOO) (NDIABO 02ml) and the 4th diabetic group receives (02 ml) of olive oil (DIABO 02ml).

The doses of olive oil were administered twice daily, in the morning and afternoon, during the 38 days of the experiment. Weekly body weights were measured with a "KERN" scale and blood glucose was measured using an Accu check blood glucose meter throughout the experimental period. At the time of sacrifice, the livers and kidneys of the rats were removed, rinsed with physiological saline and dried. The organs were weighed immediately.

Analysis of blood parameters

At the end of the experimental period, the rats were sacrificed after an overnight fast. Blood samples were immediately collected in previously labeled tubes. After centrifugation at 3500 rpm for 10 min, the serum obtained will be used for assays of serum glucose (SG), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol levels (HDLc) the analyzes were performed spectrophotometrically, according to the specifications of the supplier of the standard SPINREACT kits.

Statistical analysis of the results

The results were presented on average plus or minus the standard deviation (mean ± SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test using GraphPad Prism 5. The level of significance was: significant when $P \leq 0, 05$; highly significant when $P \leq 0.01$ and very highly significant when $P \leq 0.001$.

Results and discussion

Fatty acid composition

The fatty acid composition of the olive oil is shown in Table 1. There are three main types of fatty acids that can be present in a triglyceride, SAFA (Cn: 0), monounsaturated, MUFA (Cn: 1) and polyunsaturated, (PUFA) with two or three double bonds (Cn: 2, 3).

Table 1. fatty acid composition of the oil.

Fatty acid	(%)/total fatty acid	Standards IOC
Palmiticacid (C16:0)	14,40	07,50 – 20,00
Palmitoleicacid (C16:1)	00,91	00,30 – 03,50
Stéaricacid (C18:0)	01,96	00,50 – 05,00
Oleicacid (C18:1)	81,14	55,00 – 83,00
Linoleicacid (C 18:2)	01,59	02,50 – 21,00
SAFA	16,36	
MUFA	82,05	
PUFA	01,59	

SAFA refers to Saturated Fatty Acids;
MUFA, Monounsaturated Fatty Acids
PUFA, Polyunsaturated Fatty Acids.

The results obtained show that the fatty acid composition of this olive oil studied meets the standards set by the International Olive Council (IOC, 2001). It is rich in oleic acid (C18: 1, ω9) with a percentage of (81.14%) this percentage is higher than for other varieties Algerian, Limli, Blanquette and Bouricha (Benrachou *et al.*, 2010).

Prospective studies (Jacotot, 1997) show that a predominantly MUFA diet is associated with an improvement in glycemic equilibrium as assessed by glycemia, glycosuria, and glycated hemoglobin with a decrease in insulin requirements. The lipid profile is also improved. A study by Madigan (Madigan, 2000) compares the effects of a diet rich in PUFA with a diet rich in MUFA in 11 patients with type 2 diabetes for 2 weeks.

The results show a rise in glucose and insulin levels in the blood following the high linoleic acid diet compared to a diet rich in oleic acid. Thus, an oleic acid diet should be preferred over a diet rich in polyunsaturated fatty acids in the type 2 diabetic patient.

Mancini's research group (Mancini, 1997) has shown that a diet rich in olive oil, low in saturated fat, moderately rich in carbohydrates and providing soluble fiber provided by fruits, vegetables and cereals is the best dietary solution in type 2 diabetes.

It improves glycemic control and increases insulin sensitivity. The fasting glucose results showed that the higher the olive oil consumption, the lower the fasting glucose level.

It has also shown that in non-insulin-dependent diabetics, a diet rich in MUFA and low in complex carbohydrates significantly lowers blood glucose, insulinemia and postprandial triglyceridemia compared to a diet low in MUFA and rich in complex carbohydrates. Thus, Mancini states that it is therefore clearly not possible to recommend to non-insulin-dependent diabetics a diet low in fat.

On the contrary, he could ask these patients to replace carbohydrates with olive oil (Henry, 2003).

Otherwise, the percentage of linoleic acid (C 18: 2 ω 6) is 01.59% is sufficient to prevent a deficiency of essential fatty acid (Lapillone *et al*., 2003).

On the other hand, this oil contains saturated fatty acids represented by Palmitic acid C 16: 0 (14.40%) and stearic acid C 18: 0 (1.96%). It should be noted that health authorities around the world are encouraging people to reduce the consumption of saturated fatty acids, because of numerous studies that have shown that they can increase the "bad" cholesterol and consequently a higher risk of diseases cardiovascular (American Heart Association, 2018).

Body weights

The results obtained are shown in Fig.1.

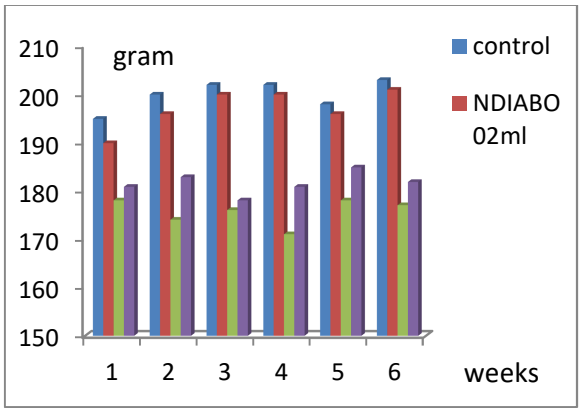

Fig. 1. Body weight monitoring during the experimentation period.

These results show an increase in the body weight of the rats of the control (Control) and non-diabetic rats treated with the 2ml dose (NDIABO) related to the normal growth of these animals as well as a reduction of diabetic weights (DIAB) which would be related to metabolic disorders due to diabetes. Lack of insulin activates lipolysis in adipose tissue and causes weight loss (Prabhakar *et al*., 2008). An increase in the weight of the diabetic groups treated with the 2ml dose (DIABO) was observed, but it remains lower than that observed in the control rats (Control). This suggests that olive oil exerted a slight effect on the insulin activity of the pancreas, resulting in a slight lipogenesis which allowed the recovery of rat body weights; this weight gain is also related to the normal growth of rats (Davis, 2004).

Relative organ weights

The results of the relative weights of organs (liver and kidney) obtained are presented in Table 2.

Table 2. Relative weight of organs (liver and kidney).

Groups	Relative liver weight	Relative kidney weight
Control	5,15 ± 0,17	0,99 ± 0,05
DIAB	6,85 ± 1,08	1,35 ± 0,38
NDIABO 02 ml	6,05 ± 1,08	0,99 ± 0,18
DIABO 02 ml	6,65 ± 0,39	1,24 ± 0,06

The previous results show that the relative weights of kidneys and livers of diabetic rats (DIAB) are higher compared to the rats of batches (Control) and (NDIABO).

The liver plays a major role in the metabolic regulation of dietary nutrients, including carbohydrates and lipids (Mezey *et al.*, 1999), and the increase in relative organ weights can be considered as an indicator of the toxicity of substances used, in this case streptozotocin. Our results are consistent with the bibliographic data of (Horiguchi *et al.*, 1996, Asagba *et al.*, 2002). Several studies show that animals fed on PUFA-rich diets reduce liver fat accumulation compared to diets rich in saturated fatty acids (Crescenzo *et al.*, 2012).

In this regard, Bjermoand al (Bjermo *et al.*, 2012) indicate that a 10-week PUFA-rich isocaloric diet reduces the fat content of the liver and tends to reduce insulin resistance compared to a diet rich in saturated fatty acids in people with abdominal obesity and diabetes type 2.

Blood samplesanalysis

Fastingglycemic

Fasting blood glucose measurements taken during the treatment period are shown in Fig.2.

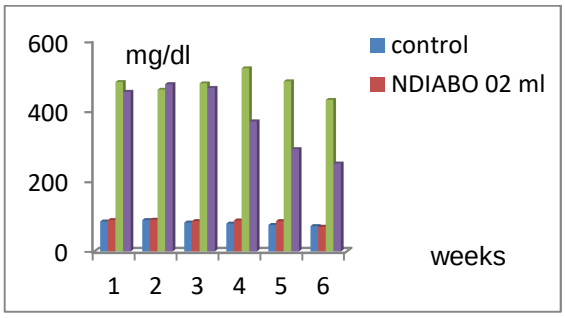

Fig. 2. blood glucose during the experimental period.

The "control" group shows no change in blood glucose concentrations for 38 days of experience. However, for the diabetic group (DIAB), an increase in blood glucose levels was observed during this period. There was also a significant decrease in blood glucose from week 4 in the diabetic lot treated with olive oil (DIABO).

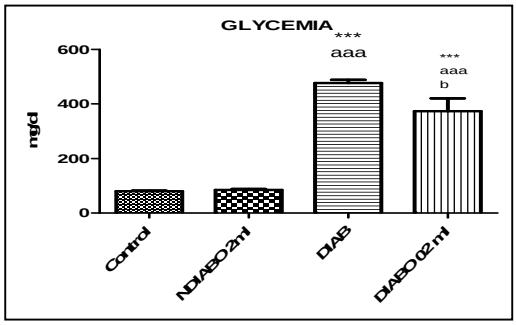

Fig. 3. Comparison of fasting glucose averages.

Statistical results obtained using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests (Fig. 3), show that the fasting glucose levels of DIAB and DIABO rats are higher in these lots compared to control lots and NDIABO with a very highly significant difference ($p \leq 0.001$). Similarly, there is a significant decrease ($P \leq 0.05$) between the fasting blood glucose levels of the DIAB and DIABO lots, but the blood glucose levels of DIABO remain higher than that of the control lot; the period of treatment and the dose used did not normalize blood glucose.

From these results it can be deduced that the increase in fasting blood glucose in diabetic lots is related to the diabetic effect of streptozotocin on pancreatic cells (Junod *et al.*, 1967) and lack of treatment of rats. (Guillausseau *et al.*, 2003).

The extra virgin olive oil treatment improved the fasting blood glucose level by causing a decrease in the DIABO lot compared to the NDIABO lot; this result is in agreement with those obtained by Violiand al (Violi *et al.*, 2015). But this treatment and its duration did not allow a normalization of this glycemia. This decline could be related to the richness of olive oil in antioxidants such as tocopherols (Psomiadou *et al.*, 2000) and polyphenols (Visioli and Bernardini, 2011). According to Schuler (Schuler, 1990), the antioxidant activity of tocopherols, mainly vitamin E, which may have beneficial effects on oxidative stress-related diseases through various mechanisms (Devaraj *et al.*, 1999).

The mechanism of the hypoglycaemic effect of natural antioxidants may be due to the potentiation of insulin release, increased peripheral glucose uptake and attenuation of oxidative stress as well as improved antioxidant defenses of the body (Wainstein *et al.*, 2012).

Impact on the lipid profile.

Cholesterol and triglycerides as many of the basic elements and components of the body. Abnormal levels generally occur due to defects in the synthesis, degradation and transport of their associated lipoprotein particles (Mensink *et al.*, 2003).

Total Cholesterol

The cholesterol levels after sacrifice are shown in Fig.4.

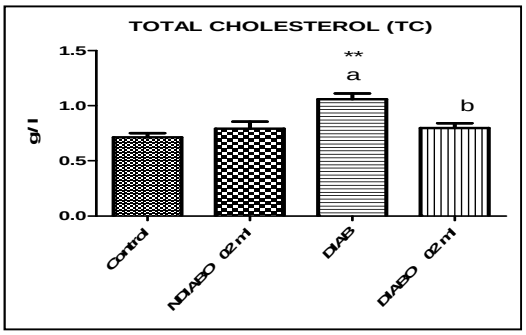

Fig. 4. Evaluation of total cholesterol levels (TC) (g/l).

The results, using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests (Fig.4), show that cholesterol levels in DIAB rats are higher in this lot compared to batches. Control and NDIABO with a highly significant difference ($p \leq 0.01$). Similarly, there is a significant decrease ($P \leq 0.05$) between the cholesterol levels of DIABO and DIAB, but the cholesterol levels of DIABO remain slightly higher than those of the control group.

The consumption of HOEV improved the total cholesterol by causing a decrease in the rates in the DIABO lot compared to the DIAB lot; this result is consistent with that of Mattson *et al*, Kris-Etherton (Mattson and Grundy, 1985; Kris-Etherton *et al.*, 1999) who showed that a diet rich in monounsaturated fatty acids decreased plasma cholesterol. This effect could be related to the quantities of phytosterols brought by this diet rich in extra virgin olive oil (Pelletier and al, 1995).

Triglycerides

The triglyceride (TG) levels after sacrifice are shown in Fig. 5.

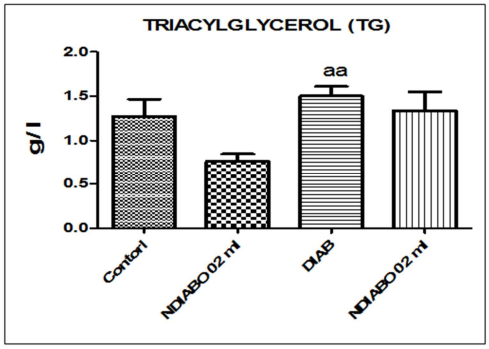

Fig. 5. Evaluation of triacylglycerol (TG) levels (g / l).

The results obtained, using the one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests (Fig. 5), show that the triglyceride (TG) levels of the batch of DIAB rats are higher compared to lot DIABO with a highly significant difference ($p \leq 0,01$) with a non-significant difference with the control batch and NDIABO.

In our study, olive oil is associated with a nonsignificant reduction in TG, consistent with studies by Kris-Etherton and al, Berglund and Allman-Farinelli (Kris-Etherton *et al.*, 1999; Berglund, 2007; Allman-Farinelli, 2005) but disagree with other authors who have shown no significant changes in lipid profiles (Madigan, 2000; Ródenas *et al.*, 2005) while Chang and Huang found that TG increased after a high monounsaturated diet (Chang and Huang, 1990).

The increased consumption of MUFA improves insulin sensitivity and therefore reduces TG levels as lipoprotein lipase, the enzyme responsible for plasma TG degradation, is an insulin-sensitive enzyme (Berry, 1997).

HDLc

The levels of HDLc after sacrifice are shown in Fig.6.

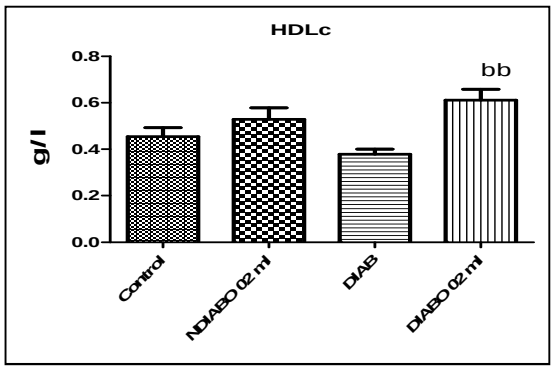

Fig. 6. Evaluation of HDLc levels (g / l).

Using the one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests (Fig.6), the results show that the HDLc levels of DIABO rats are higher than that of control and NDIABO batches with a non-significant difference and with a highly significant difference ($p \leq 0.01$) compared to the DIAB lot.

Indeed, olive oil may reduce LDL levels (Mensink and Katan, 1992) and increase HDLc levels (Mattson and Grundy, 1985). Olive oil, which contains a high level (70% - 80%) of oleic acid, is responsible for reducing triglycerides and increasing HDLc (Covas, 2008).

The decrease in HDLc is a major risk factor for cardiovascular disease. Conversely, each 1% increase in this "good" cholesterol reduces the coronary risk by 3%. The existence of an inversely proportional relationship between the plasma concentration of HDLc and the occurrence of myocardial infarction, especially in women (Henry, 2003).

Extra virgin olive oil of the "Rougette" variety could be effective in the treatment of diabetes by significantly lowering fasting blood glucose and preventing cardiovascular disease by decreasing total cholesterol and increasing HDLc.

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