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Original Article Research

EFFECT OF VIRGIN OLIVE OIL SUPPLEMENTATION ON LIPID PROFILE AND OXIDATIVE STATUS IN RATS

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ABSTRACT

Abstract

The aim of the present study is to investigate the effect of virgin olive oil on some blood parameters in male Albino rats supplemented with normal diet. thirty male Sprague Dawley rats, (90-110 g), were used in the present study, and were divided into three groups (10 in each), 1st group (control), received basal diet and supplemented with 1ml saline. 2nd and 3rd groups received basal diet, and supplemented daily with 1ml/100 gm B.W and 2ml/100 gm B.W of virgin olive oil (VOO), respectively for 4 weeks. Blood samples were collected weekly from all rats. Serum samples was obtained for assay of lipid profile levels and hepatic lipid peroxidation (MDA) enzyme. Blood lysate was used for antioxidant enzymes activities SOD, GPx and CAT.

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Introduction:

Olive (Olea europaea L.) oil is a fundamental component of the Mediterranean diet (Zhang *et al.,* 2013). In the last few decades there has been a significant increase in the global consumption of olive oil, even in countries where it is not produced, such as Canada and Japan (Mili, 2006). This is due in large part to its nutritional and health promoting effects (Solyanik *et al*., 2004), which have been related to the optimal balance between saturated, monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), as well as to minor components such as chlorophyll, polyphenols and tocopherols (Lazzez *et al*., 2008).
inflammatory process (Wable *et al*., 2004).

Vegetable oils have been historically present in many food stuffs and health care products. They play a leading role in human nutrition, and a source of many essential nutrients. Vegetable oils are generally obtained from the seeds of plants like soya bean, sunflower, rape, palm, peanut and corn. Nevertheless, the importance of olive oil, obtained from a drupe fruit, is increasing due to the biological properties of several of its components that preserve health and prevent many degenerative illnesses (Mackenbach, 2007). Effectively, olive oil has a beneficial effect in controlling blood pressure and improving the immune function by attenuating the
The second and third group received basal diet, and

Many studies have been conducted to prove its potential through oil, whole fruit and leaf extract as anticancer, antimicrobial and antiviral effects (Covas, 2007 and Awney, 2010). Since olive oil is a wild oil commonly available in the world and especially in the Mediterranean and its leaves are used in folk medicine for treatment, it is therefore deemed interesting to examine the effect of virgin olive oil supplementation on lipid profile (TG, TC, LDL-C, VLDL-C, HDL-C and AI) parameters, glucose level and on oxidative status in albino rats, after oral gavages of different doses of virgin olive oil for a period of 30 days.

MATERIAL AND METHODS

Olive Oil:

oil in the present study was obtained from olive (Olea europaea; family Oleaceae), a traditional tree crop of Tarhuna city farms, Libya. The oil was identified by Dr. Salem M. Abd-Alsadiq. Senior Botanist, Department of Crops and Horticulture - Faculty of Agriculture – Tripoli University. Tripoli-Libya. Olive oil was administered in two doses by gastric tube for 4 weeks: Low dose (1 ml / 100g B.W) olive oil and high dose (2 ml / 100g B.W) olive oil (Nandakumaran *et al.,* 2012).

Animals:

The study was conducted in the Animal House of National Research Centre (NRC), Cairo, Egypt. Thirty Adult male rats (Sprague Dawley Strain) weighing between 90-110 g were used for the study. The animals used for the study were randomly selected. All rats were active, apparently healthy and free from abnormalities and disease and housed in commercial cages, equipped with automatic drinkers and feeders, at room temperature maintained at 25 °C, with alternating 12 hour light 12 hour dark cycle. The animals were kept for 10 days for acclimatization before the experiment.

Feeding regimen:

Basal diets were formulated to cover the requirements of rats as recommended in NRC (1977). Diets were subjected to chemical analysis according to AOAC (2012).

Experimental design:

The rats were equally and randomly divided into three groups (10 in each): The first group was considered as control group, and received basel diet with 1ml saline by gastric tube daily for 4 weeks.
(1984), and expressed as U/mg.

supplemented with either 1ml/100g or 2ml/100g of virgin olive oil (VOO), respectively, administered by gastric tube for 4 weeks.

Blood collection and serum separation:

Blood samples were collected individually by orbital venus plexus technique under mild ether inhalation anaesthesia. Samples were obtained at the early morning before access to feed and water at the end of every week. Portion of blood samples was collected into heparinized tube for antioxidant parameters in whole blood cell lysate. The other portion of blood samples was collected into plain tubes and allowed to coagulate at room temperature and centrifuged at 1000 g for 20 min to obtain sera. The clear, non-haemolysed supernatant sera were quickly collected for each animal and stored at -20 °C for lipid profile and glucose.

Estimation of lipid profile and glucose levels:

Serum TC, TG and HDL-C levels were estimated colorimetrically using commercial reagent kits (Spectrum Diagnostic, Egypt) and expressed as mg/dl.

Serum Low Density Lipoproteins cholesterol (LDL-C) level was calculated according to the formula developed by Friedewald *et al.* (1972) using the following equation:

$$\text{Serum LDL-c} = \text{TC} - (\text{HDL-c} + \text{TGs} / 5)$$

Atherogenic index (AIX) was calculated according to the formula adopted by Hostmark *et al.* (1991), as follows: Atherogenic index = (TC - HDL-c) / HDL-c.

Serum glucose concentration: Was determined by enzymatic method explained by Trinder (1969), and expressed as mg/dl.

Estimation of antioxidants and oxidative markers:

Superoxide dismutase activity (SOD): was determined in blood cell lysate, according to the method described by Jewett and Rocklin (1993), and expressed as U/mg.

Glutathione peroxidase activity (GPx): was determined in blood cell lysate, according to method dscribed by Paglia and Valentine (1967), and expressed as U/mg.

Catlase enzyme activity (CAT): was determined in blood cell lysate, according to the method of Aebi, group. Moreover, the values indicated significant

Malondiadehyde (MDA): Hepatic lipid peroxidation was determined in serum according to method Drapper and Hadley (1990), and expressed as nmol/ml.

StatisticaI AnaIysis:

All data are expressed as Means±SE and statistical analysis according to **Snedecor and Cochran (1980)**. was done using SPSS statistical package. Means were compared by the least significance difference test at 5% level of probability (Two way anova test).

Table (1): Fatty acid composition of dietary olive oil:

Fatty acid	g/100g
Palmitic acid	10.28
Palmitoleic acid	0.77
Stearic acid	3.39
Oleic acid	64.80
Lenoleic acid	14.34
Lenolenic acid	0.64
Archidonic acid	0.74
Gadoleic acid	0.62
Behenic acid	2.84
SFA	17.28
MUFA	66.20
PUF	14.99

SFA: saturated fatty acids;
MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

Results:

Effect of virgin olive oil on lipid profile and glucose in rats: The present study investigates the supplementation of two different doses of virgin olove oil; (low dose of virgin olive oil (LVOO) and high dose of virgin olive oil (HVOO) on lipid profile, glucose, antioxidant and oxidative stress marker in rats. In the present study, rats supplemented with with either low dose of virgin olive oil (LVOO) or high dose of virgin olive oil (HVOO) showed significantly decreased serum TG, TC, LDL-C, VLDL-C, AI and glucose levels during 1st, 2nd, 3rd, and 4th weeks of the experimental period, when compared to the basel diet decrease of serum TG, TC, LDL-C, VLDL-C, AI and glucose in rats supplemented with HVOO, during 1st, 2nd, 3rd and 4th weeks of the experimental period, when compared with rats supplemented to LVOO group.

On the contrary the results recorded in table (2) for HDL-C levels, showed significant increase in rats supplemented with LVOO, during 1st, 2nd, 3rd and 4th weeks of the experimental period when compared with BD group. Moreover, values indicated significant increase of HDL-C of rats supplemented with HVOO, during 1st, 2nd, 3rd and 4th weeks of the experimental period, when compared with rats supplemented with LVOO group.

Effect of virgin olive oil on antioxidant parameters in rats:

Data tabulated in (fig. 1) showed that rats fed basel diet and supplemented with low dose of virgin olive oil (LVOO) exhibited significant increase in the activites of SOD, GP_X and CAT during 1st, 2nd, 3rd and 4th weeks of the experimental period, when compared with BD group. Moreover, groups supplemented with HVOO, showed significant increase in SOD, GP_X and CAT activites, during 1st, 2nd, 3rd, and 4th weeks of the experimental period, when compared with BD group. Furthermore, the present results showed that rats supplemented with HVOO, caused significant improvement in serum SOD, GP_X and CAT activites values during 1st, 2nd, 3rd, and 4th weeks of the experimental period, when compared with rats supplemented with LVOO group.

On the contrary, results in (fig. 4) recorded for serum MDA values showed significant decrease in rats fed basel diet and supplemented with LVOO, during 1st „ 2nd, 3rd and 4th weeks of the experimental period, when compared with BD group. Moreover, groups supplemented with HVOO, showed significant decrease in MDA values, during 1st, 2nd, 3rd, and 4th weeks of the experimental period, when compared with BD group. Furthermore, the present results showed that rats supplemented with HVOO, caused significant decrease in serum MDA values during 1st, 2nd, 3rd, and 4th weeks of the experimental period, when compared with rats supplemented with LVOO group.

Table2: Effect of Virgin olive oil supplementation on serum lipid profile and glucose levels in rats.

Groups	Parameters	BD	LVOO	HVOO	LSD
TG (mg/dl)	I	2.80±152.20	150.60±0.65	147.40±0.80	0.87
	II	3.27±154.80	148.10±0.20	144.30±0.60	
	III	2.38±157.20	143.60±0.62	141.90±0.20	
	IV	1.67±159.60	141.40±0.45	136.10± 0.26	
TC (mg/dl)	I	3.33±78.65	74.00±3.50	71.25±1.33	2.15
	II	2.00±81.46	73.50±1.74	70.46±1.50	
	III	2.77±83.83	72.60±1.31	69.85±0.56	
	IV	3.89±85.86	71.80±0.26	69.15± 0.43	
HDL-C (mg/dl)	I	1.81±24.00	25.80±1.70	27.50±1.65	1.29
	II	1.04±27.60	29.50±0.26	31.70±0.62	
	III	1.58±29.00	31.00±0.30	32.80±0.36	
	IV	1.92±29.80	31.60±0.45	33.70±0.30	
LDL-C (mg/dl)	I	1.65± 24.21	18.08±1.13	14.27±0.29	1.98
	II	1.97±22.90	14.38±1.59	9.90±0.95	
	III	1.63±23.39	12.88±1.03	8.67±0.85	
	IV	1.93±24.14	11.92±1.45	8.23±0.74	
VLDL-C (mg/dl)	I	0.45±30.44	30.12±0.13	29.48±0.16	0.17
	II	0.65±30.96	29.62±0.08	28.86±0.12	
	III	0.47±31.44	28.72±0.12	28.38±0.14	
	IV	0.33±31.92	28.28±0.09	27.22±0.10	
AI (mg/dl)	I	0.61±2.27	1.86±0.17	1.59±0.13	0.13
	II	0.28±1.95	1.49±0.13	1.22±0.10	
	III	0.20±1.89	1.34±0.19	1.12±0.04	
	IV	0.27±1.88	1.27±0.17	1.05±0.03	
Glucose (mg/dl)	I	91.00±0.18	89.49±0.08	88.60±0.68	0.79
	II	90.20±0.09	89.00±0.16	87.47±0.39	
	III	89.40±0.15	85.85±0.21	84.65±0.45	
	IV	89.50±0.32	84.95±0.18	84.14±0.34	
ANOVA			P≤ 0.05		

Data indicate mean ± standard error at (p≤ 0.05), N= 10 rats, BD= Control, LVOO= low dose of virgin olive oil, HVOO = High dose of virgin olive oil , I= 1st week, II= 2nd week, III= 3rd week, IV= 4th week, LSD= (Least significant difference). TG= Triglyceride, TC= Total cholesterol, HDL-C= High density lipoproteins cholesterol, LDL-C= Low density lipoproteins cholesterol, VLDL-C= Very low density lipoproteins cholesterol, AI=Atherogenic index.

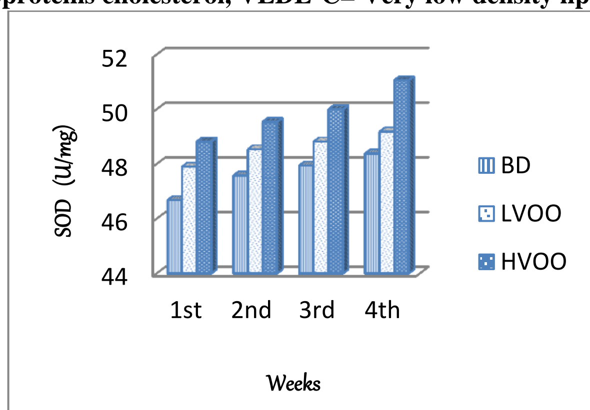

Fig.1: Effect of olive oil supplementation on SOD in blood cell lysate of rats. BD= Control, LVOO = low dose of virgin olive oil, HVOO = high dose of virgin olive oil. SOD = Superoxide dismutase.

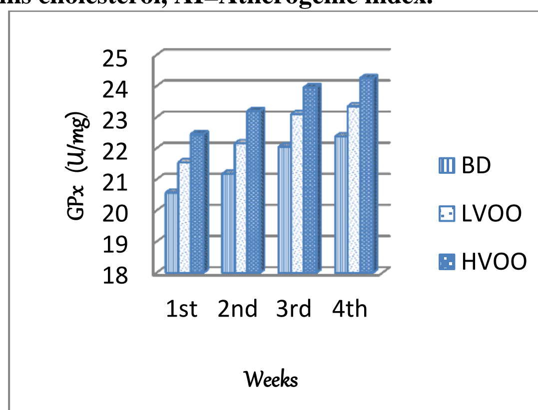

Fig.2: Effect of olive oil supplementation on GP_X in blood cell lysate of rats. BD= Control, LVOO = low dose of virgin olive oil, HVOO = high dose of virgin olive oil. GP_X = Glutathione peroxidase.

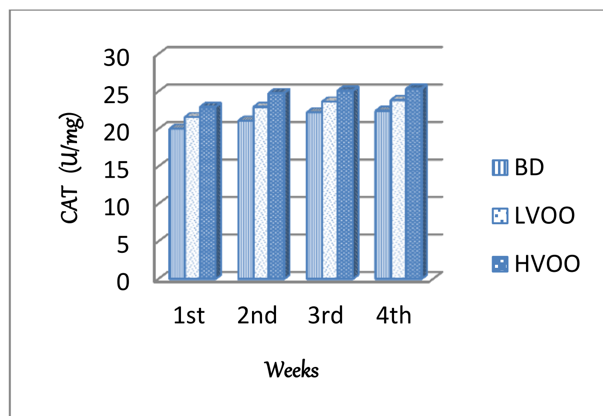

Fig.3: (18 c): Effect of olive oil supplementation on CAT in blood cell lysate of rats. BD= Control, LVOO = low dose of virgin olive oil, HVOO = high dose of virgin olive oil. CAT = Catalase.

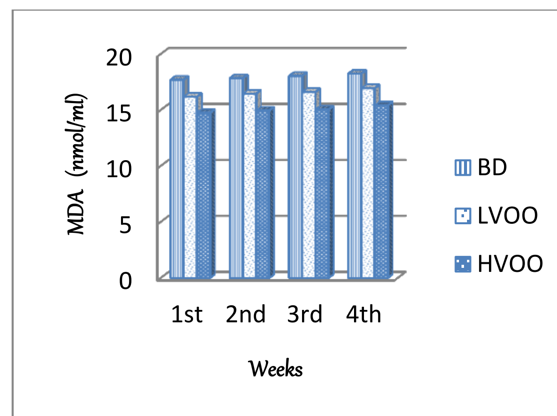

Fig.4: Effect of olive oil supplementation on MDA in serum of rats. BD= Control, LVOO = low dose of virgin olive oil, HVOO = high dose of virgin olive oil. MDA = Malondialdehde.

Discussion:

The present study is an attempt to assess the hepatoprotective potential with either low dose of virgin olive oil (LVOO) and high dose of virgin olive oil (HVOO) in rats. The results of the present study showed that the oral supplementation of either LVOO or HVOO in rats caused significant decrease in the serum TG, TC, LDL-C, VLDL-C, AI and glucose. Meanwhile; a significant increase was seen in HDL-C values at 1st week from the beginning of the experimnt. The possible explantation of these observed reduction may be attributed to the healthy effects of VOO on cardiovascular risk factors which have been attributed to its high content of MUFAs, such as oleic acid. In this context, MUFAs are suggested to be effective in improving serum lipid profile levels, through a decrease in TG, TC, LDL-C, VLDL-C and AI with increase in HDL-C. All former results confirm the finding of Moreno and Mitjavila, (2003); Perona *et al.* (2006); Rosa casas *et al.* (2017); Elias *et al.* (2017) and Khan *et al.* (2017), they reported that olive oil product reduced serum TG, TC, LDL-C, VLDL-C, AI and glucose levels. in addition Massimo *et al.* (2009) who suggested that the healthy effect of olive oil referd to MUFAs that may play role in modulate atherosclerosis by affecting vascular endothelium, through increasing the amount of oleic acid in the arterial wall and displacing saturated fatty acids (SFAs), while leaving polyunsaturated fatty acid (PUFAs). Thus, profile levels, through a decrease in TG, TC, LDL-C, VLDL-C and AI and increase HDL-C. Besides olive oil has been shown to lower blood glucose levels (Tahvonen *et al.*, 2005). This confirmed the improvement of blood lipids in the present study. Other components of olive oil such as oleic acid; a compound which belong to the class of MUFA, has a beneficial effect in the reduction of glycemic load which might be increased insulin sensitivity (Tahvonen *et al.*, 2005; Schwingshacki and Hoffmann, 2014; Qian *et al.*, 2016 and Elias *et al.*, 2017).

In this study, rats supplemented with either low dose of virgin olive oil (LVOO) or high dose of virgin olive oil (HVOO), exhibited significant increase in the activites of SOD, GP_X and CAT. Meanwhile; the results recorded for Serum MDA values showed significant decrease in rats supplemented with either low dose or high dose of virgin olive oil, when compared with control group. Through this increase in antioxidant enzyme activity, the high dose of virgin olive oil (HVOO) showed the best antioxidant enzyme activities. The mechanism proposed to explain the positive effect of HVOO may be attributed to its richness in MUFA, mainly oleic acid which has different effects on lipid profile levels and peroxidation in rabbit hepatic mitochondria (Ochoa-Herrera *et al.,* 2001). However, the obtained data showed that HVOO was more effective than LVOO in induced oxidative stress in the liver. In healthy humans, the short-term consumption of olive oil decreased serum oxidative stress (Weinbrenner *et al.,* 2004) and their lipoprotein

olic acid may contribute in improving serum lipid acid and resistant to oxidation (Sola *et al*., 1997).

Moreover, PUFAs are more susceptible to peroxidation resulting in MDA formation (Esterbauer *et al.,* 1991). Because of their peculiar structure that is the presence of one or more double bonds-UFA are more susceptible to free radical damage and thus could increase the susceptibility of LDL particles to oxidation. Most of studies comparing the effects of a MUFA-rich diet with PUFA-rich diet on LDL oxidation parameters have found a higher resistance of LDL particles to oxidation after the consumption of MUFA-rich diet (Kratz *et al*., 2002). The heathy effects of the dietary MUFA, including lower endothelial activation (Massaro *et al*., 2002) and susceptibility of LDL to oxidation (Aguilera *et al.,* 2004) are indeed to be considered.

Conclusion:

The results of the present study showed that virgin olive oil improved antioxidant enzymes activities by preventing excessive lipid peroxidation to increase MUFA composition and by improvement of serum lipid profiles and glucose levels.

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