Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8648
Doi: 10.4025/actascihealthsci.v44i1.58558

The beneficial effects of virgin olive oil against oxidative stress induced by hypercholesterolemia in rats

Aicha Kribeche* **and Tayeb Idoui**

Laboratory of Biotechnology, Environment and Health, University of Jijel, Jijel, 18000, Algeria. *Author for correspondence. E-mail: kriaicha@yahoo.fr

ABSTRACT. Cardiovascular disease (CVD) remains the major cause of mortality in the world, typically claiming a third of all deaths. The primary cause of CVD is atherosclerosis. Therefore, timely prevention and therapy of atherosclerosis are able to reduce the risk of the development of its clinical manifestations. Anti-atherosclerotic activity of medicinal plants mainly appears in their multiple effects. This study was carried out to evaluate the hypolipidemic activity of virgin olive oil in experimentally induced hyperlipemic Wistar. A total of 24 rats were randomly allocated to 4 equal groups and treated as follows for 50 days: (1) Normal control (NC); that were fed with a standart diet; (2) High Cholesterol Diet Control (HCD); which received high cholesterol diet for 50 days; (3) Animals receiving high cholesterol diet for 50 days, after this period the animals are fed for eight days by the standard food and receiving by gavage virgin olive oil (HCD+VOO) and (4) Animals fed for eight days with the standard food and receiving by gavage olive oil (VOO). High Cholesterol Diet containing yolk egg and coconut oil. Results showed that olive oil caused a significant ($p < 0.01$) reduction in serum levels of Total Cholesterol (TC), Triglycerides (TG), Low–Density Lipoprotein Cholesterol (LDL) and Atherogenic Index Serum (AIS). The results also demonstrated a significant ($p < 0.01$) increase in High–Density Lipoprotein Cholesterol (HDL). Moreover, virgin olive oil induced a significant reduction in liver lipid content. On the other hand, a High cholesterol diet induced oxidative stress was measured by estimating reduced glutathione level and amount of thiobarbituric acid reactive substances (TBARS) formed as an index of lipid peroxidation in a liver and a heart. Virgin olive oil supplementation attenuated all these variations. Our observations of the study indicate that the virgin olive oil has a significant antihyperlipidemic potential.

Keywords: high cholesterol diet; LDL cholesterol; virgin olive oil; oxidative stress, rats.

Received on April6, 2021.
Accepted on July9, 2021

Introduction

Atherosclerosis, the underlying cause of cardiac ischemia, heart failure, heart attack, stroke, and peripheral vascular disease, is known to be one of the major causes of death and morbidity worldwide (Kim & Shim, 2019). There are a number of genetic, metabolic, and environmental factors involved in the formation and evolution of the atherosclerotic plaque (Markin, Sobenin, Grechko, Zhang, & Orekhov, 2020). A well-known risk factor in humans is hypercholesterolemia, i.e., elevated total cholesterol (TC) and low-density lipoprotein cholesterol (LDL) (Pourrajab et al., 2021), and other important contributors to this disease include inflammation, oxidative stress and insulin resistance (Subramani et al., 2017). The accumulation of LDL in intima causes oxidation states by increased levels of reactive oxygen species or oxidative enzymes released by inflammatory cells. These oxidized lipids give rise to over expression of adhesion molecules and secretion of pro-inflammatory cytokines that promote generation of fatty streaks made of T cells and foam cells loaded with lipids (Namazi, Shomali, Taghikhani, & Nazifi, 2018).

For the treatment of the atheroscleosis, statins are commonly used due to their excellent efficacy in minimizing the LDL level in the serum and inhibiting the vascular risk, but the continuous use of statins lead to more side effects viz., rhabdomyolysis, myopathy, liver injury, muscle toxicity and acute renal failure (Hopewell, Reith, & Armitage, 2014; Jose, Al-Tamimi, Helal, Jimmy, & Al Riyami, 2014; Diao, Sun, Ma, Li, & Wang, 2018).

Olive oil is the primary source of fat in the Mediterranean diet. A high degree of adherence to the traditional Mediterranean diet has been associated with a reduced risk of overall and cardiovascular mortality, cancer incidence and mortality, and incidence of Parkinson and Alzheimer disease (Roman,Jackson, Gadhia, Roman, & Reis, 2017).

This study was carried out to evaluate the hypolipidemic activity of olive oil in Wistar rats affected by experimental hyperlipidemic induced by the intake of high levels of saturated fat and yolk egg. Administration of high-fat diets to promote atherosclerosis in animal models is a valuable tool for studying pathogenesis and testing novel compounds for treatment or prevention of atherosclerosis (Namazi et al., 2018).

Over the past 150 years, there have been numerous efforts to explain the complex events associated with the development of atherosclerosis. This study investigated the hypothesis that oxidative stress is a pivotal feature of the atherogenesis, and can induce alterations in blood lipid profile and antioxidant. The study tested whether hyperlipidemia induced changes in lipid profile and antioxidant status and evaluate the effect of olive oil on plasma lipid and lipoprotein composition and on LDL oxidation susceptibility as markers of atherosclerosis risk.

Material and methods

Virgin olive oil

The olive oil used in this study was chosen as the best from twelve other samples collected from different locations in the North-Eastern of Algeria (Jijel, Algeria) and can be considered as appropriate sources of bioactive phytochemicals. The composition was summarized in Table 1.

Table 1. Characteristics and composition of virgin olive oil used in the dietary intervention study.

Characteristics and constituents	Virgin olive oil
pH	7.68
Acidity (%)	0.7 ± 0.14
Peroxide value (meq d'O_2Kg^{-1})	1.6 ± 0.45
Iode value (g d'iode 100 g^{-1} d'huile)	87.56 ± 1.796
Fatty acids (%)	-
Oleic acid	61.19
Palmitic acid	27.67
Linoleic acid	5.09
Stearic acid	2.96
Palmitoléic acid	2.99
Total polyphenols (µg EAGmg^{-1} of extract)	107.31 ± 1.90
Flavonoids (µg EQmg^{-1} of extract)	0.473 ± 0.0014
Antioxidant activity (DPPH scavenging %)	80.46± 1,58
Sensorial score	3.5

Animals and experimental design

The experiment was conducted on 24 male Wistar rats weighing approximately 200 g. The animals were divided into four groups of 6 rats in each. The rats were maintained in individual cages, under controlled temperature, humidity and illumination conditions, with water and diet *ad libitum* for eight weeks. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the Algerian Association for Experimental Science (AASEA) and approved by the Ethics Committee of department of Biological Science of the Jijel University.

Group 1: Normal Control (NC)

Group 2: High Cholesterol Diet Control (HCD), animals receiving an atherogenic food for 50 days

Group 3: Animals receiving high cholesterol diet for 50 days, after this period the animals are fed for eight days by the standard food and receiving by gavage virgin olive oil (1% of body weight) (HCD+VOO).

Group 4: Animals fed for eight days with the standard food and receiving by gavage virgin olive oil (1% of body weight) (VOO).

Hyperlipidemia treatment (atherogenic food)

Rats were made in hyperlipidemia condition with the method described by Metwally, El-Gellal and El-Sawaisi (2009) with minor modification, i.e. rats were given a mixture of yolk egg and coconut oil (80: 20) by oral gavage as much as 1% of body weight for 50 days. Hyperlipidemia treatment were given to group 2 (HCD) and group 3 (HCD+VOO) as described above in experimental design.

Blood sampling and biochemical determinations

The blood samples were taken through retro orbital venous plexus. Blood samples were centrifuged for ten minutes at 3000 rpm. Serum was analyzed using commercial kits (*Biomaghreb* Kit) for levels of total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol according to the protocol described by the manufacturer. The Atherogenic Index Serum (AIS) was calculated through the following formula (Balogun & Adebayo, 2007).

Atherogenic Index Serum = Total Cholesterol / HDL cholesterol.

Determination of hepatic lipid content

Liver samples were grinded and subjected to lipid extraction with chloroform: methanol (2:1) as described by Folch, Lees and Stanley (1957). Twenty grams were blended for 2 minutes with cold chloroform-methanol mixture (2:1 v v^{-1}) in proportion of 10 ml of solvent mixture per gram of tissue (except 20 ml of solvent per gram of liver tissue) in a Warring blender. The slurry was immediately filtered and the clear filtrate mixed with 0.2 of its volume of 0.03M magnesium chloride in a separator flask. The mixture was allowed to stand overnight at -5°C. Upon standing, a biphasic system was obtained, with essentially all of the tissue lipids in the lower phase and non-lipid materials in the upper phase. The lower phase was drained out and accurately measured. An aliquot was taken and evaporated in an oven at 50°C to get the weight of the total lipid. The result is expressed as a percentage (%) of liver.

Determination of malondialdehyde

Malondialdehyde (MDA) was measured according to the method described by (Sastre, Pallardó, Asuncion, & Vina, 2000). Homogenates of liver or heart were made prepared at 10% (w v^{-1}) in 0.1 mol L^{-1} Tris-HCl buffer, pH 7.4. Thiobarbituric acid 0.67% (w v^{-1}) was added to aliquots of the homogenate previously precipitated with 10% trichloroacetic acid (TCA) (w v^{-1}). Then the mixture was centrifuged, and the supernatant was heated (100°C) for 15 min. in a boiling water bath. After cooling, n-butanol was added to neutralize the mixture, and the absorbance was measured at 532 nm. The results were expressed as nmol of MDA g^{-1} tissue.

Determination of glutathione

Cellular glutathione content was measured as described by Ellman (1959). 0.5 g of liver or heart tissues was homogenized with three volumes of TCA (5%) using a grinder DOUNCE. Homogenized and centrifuged at 2000 rpm, then 50 µl of the supernatant were diluted in 10 ml of phosphate buffer (0.1M, pH = 8). Consequently, were added 20 µl of DTNB 0.01 M (acid 5, 5 '- dithiobis 2-nitrobenzoic acid) to 3 ml of the mixture dilution. The measurement of the optic density was performed at 412 nm against a control prepared in the same conditions using TCA 5%. The concentrations are expressed in mmoles of glutathione /g of tissue. They are deducted from a range of glutathione, which was prepared with the same conditions as dosage did.

Statistical analysis

Results are given as means ± standard deviations. The means were compared using one-way analysis of variance (ANOVA) followed by a t-test and p-values less than 0.05 were considered significant.

Results

Body weight

By the end of the 50 days there was a significant increase ($p < 0.05$) in body weight of HCD rats when compared with NC as presented in the Table 2. The body weight of normal control rats and HCD rats increased by 8.03 and 16.82% after 7th week respectively. While the weight of the HCD+VOO rats increased by 13.99%.

Table 2. Effect of virgin olive oil on body weight (g) of high cholesterol diet Wistar rats.

	NC	HCD	HCD+VOO	VOO
Before treatment	203.25 ± 4.11	191.4 ± 26.03	214.4 ± 17.70	207.8 ± 24.63
After 50 days	221 ± 8.22	223.6 ± 20.33*	244.4 ± 23.20	214.4 ± 28.39
After 58 days	229.75 ± 9.81	227.4 ± 22.35	240.2 ± 21.52	218 ± 30.56

Values are represented as mean ± SD, n = 6. NC: Normal Control; HCD: High Cholesterol Diet Control; HCD+VOO: High Cholesterol Diet rats received virgin olive oil; VOO: rats received virgin olive oil. *$p < 0.05$.

Estimation of biochemical parameters

The Table 3 shows the increased level of Cholesterol, TG and LDL in the HCD rats as compared with the NC, whereas HDL was significantly reduced. Although, HCD control rats received the virgin olive oil significantly ($p < 0.05$) modulated the biochemical parameters and olive oil treated rats confirmed the recovery of the declined level of HDL.

Table 3. Effect of virgin olive oil on the lipid profile of high cholesterol diet hyperlipemic Wistar rats (mML^{-1}).

Groups	TC	TG	LDL	HDL
NC	1.62 ± 0.01	1.37 ± 0.96	1.56 ± 0.42	1.39 ± 0.12
HCD	1.71 ± 0.01	1.80 ± 0.12*	2.86 ± 0.02*	1.10 ± 0.07*
HCD+VOO	1.49 ± 3.63	0.86 ± 0.54*	1.44 ± 0.04*	1.17 ± 1.21
VOO	1.37 ± 2.42	1.03 ± 0.24	1.48 ± 0.03	1.34 ± 0.21

Data are represented as mean ± SD, n = 6. One-way ANOVA test against High Cholesterol Diet Control (HCD) rats: HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride; NC: normal control; HCD: High Cholesterol Diet Control; HCD+VOO: High Cholesterol Diet rats received virgin olive oil; VOO: rats received virgin olive oil. *p < 0.05.

Atherogenic Index Serum

The Atherogenic Index Serum (AIS) of groups are summarized in Table 4. The AIS of HCD rats was significantly increased ($p < 0.05$) in comparison to NC rats. The group treated with virgin olive oil showed a reduction of AIS when compared with HCD and the value decreased from 7.025 ± 1.02 in HCD group to 3.89 ± 0.83 in HCD+VOO group, equivalent to a reduction of 80.59%.

Table 4. Effect of virgin olive oil on the atherogenic index of high cholesterol diet hyperlipemic Wistar rats.

Groups	Atherogenic Index Serum
NC	3.29 ± 0.90
HCD	7.025 ± 1.02*
HCD + VOO	3.89 ± 0.83*
VOO	3.14 ± 0.72

Values are represented as mean ± SD, n = 6. NC: Normal Control; HCD: High Cholesterol Diet Control; HCD+VOO: High Cholesterol Diet rats received virgin olive oil; VOO: rats received virgin olive oil. *p < 0.05

Malondialdehyde (MDA)

Virgin olive oil showed a strong effect on lipid peroxidation as shown in the Figure 1. A significant reducing ($p<0.05$) of malonedialdehyde (MDA) concentrations in liver and heart of animals treated with virgin olive oil daily for eight days prior to hypercholesterolimia is observed (11.58 ± 1.13 and 7.25 ± 1.54 nmol/g tissue against 20.05 ± 0.96 and 18.80 ± 1.87 $nmolg^{-1}$ tissue in liver and heart respectively).

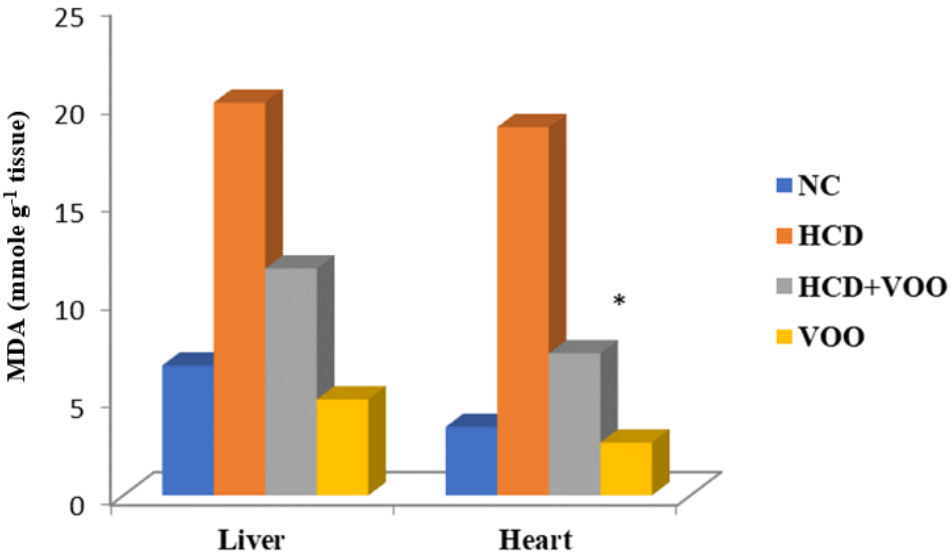

Figure 1. Effect of virgin olive oil on malondialdehyde (MDA) level of liver and heart tissue. The results are represented as mean ± SD. NC: Normal Control; HCD: High Cholesterol Diet; HCD+VOO: High Cholesterol Diet rats received virgin olive oil; VOO: rats received virgin olive oil. *p < 0.05.

Glutathione (GSH)

HCD rats show a very significant ($p < 0.01$) decrease in GSH concentrations at 50 days of treatment (0.85 ± 0.55 mM g^{-1} tissue against 1.59 ± 0.32 mM/g tissue and 1.1± 0.97 mMg^{-1} tissue against 2.90 ± 1.76 mM g^{-1} tissue for controls respectively in liver and heart) (Figure 2). The rats post-treated with virgin olive oil show a very significant increase of GSH levels compared to those HCD (1.2 ± 0.98 mM against 1.59 ± 0.89 mM and 2.90 ± 0.77 mM against 3.1 ± 1.02 mM in liver and heart respectively).

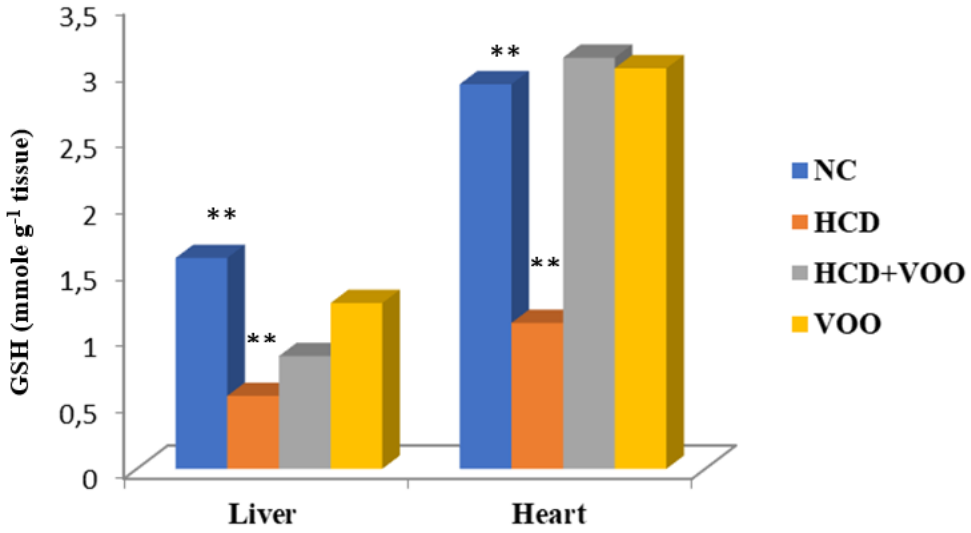

Figure 2. Effect of virgin olive oil on glutathione (GSH) level of liver and heart tissue. The results are represented as mean ± SD. NC: Normal Control; HCD: High Cholesterol Diet HCD+VOO: High Cholesterol Diet rats received virgin olive oil; VOO: rats received virgin olive oil. **p < 0.05.

Hepatic lipid fraction

The Figure 3 shows the levels of hepatic lipid of the experimental groups, hepatic lipid was significantly increase ($p<0.05$) in HCD group (47.4 ± 0.56), nevertheless, there was a significant decrease in the levels of hepatic lipid in rats received virgin olive oil (32.45 ± 0.63) when compared with HCD rats.

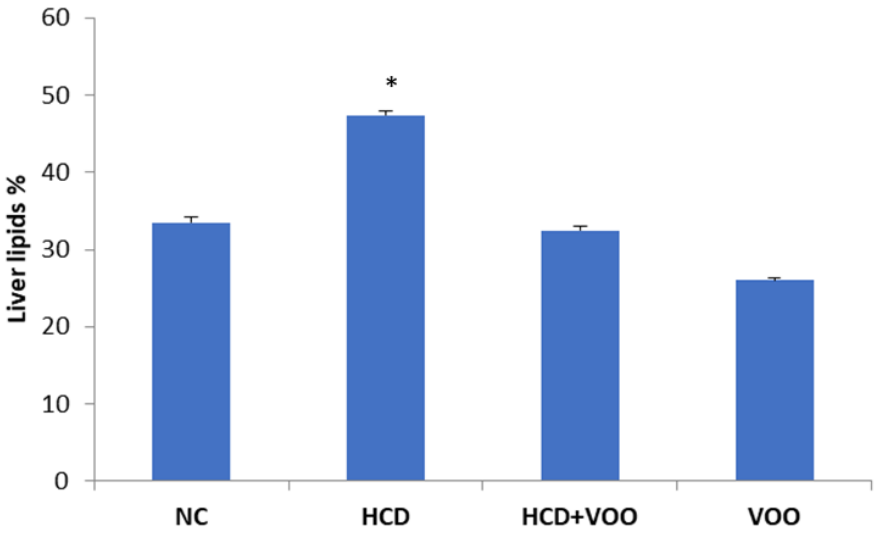

Figure 3. Effect of virgin olive oil on liver lipids. The results are represented as mean± SD. NC: Normal Control; HCD: High Cholesterol Diet; HCD+VOO: High Cholesterol Diet rats received virgin olive oil; VOO: rats received virgin olive oil. *p < 0.05.

Discussion

A high cholesterol diet has long been known to produce atherosclerosis in the aorta. For the treatment of the atherosclerosis, statins are commonly used due to their excellent efficacy in minimizing the LDL level in the serum and inhibiting the vascular risk, but the continuous use of statins lead to more side effects. A low

incidence of coronary heart disease in Mediterranean countries has been correlated with a diet that is rich in fruit, vegetables, legumes and grains (Amato et al., 2020).

This study clearly demonstrates that a high cholesterol diet (yolk egg) increased significantly the animal body weights compared to the controls ($p < 0.05$). This increase could have resulted in the increase in food and water intakes of rats. Physiologically, the increase in the appetite could be due to orexin, the stimulative hormone of appetite. Perez-Leighton, Butterick-Peterson, Billington, and Kotz (2013) proposed that throughout development of obesity, there are changes in orexinergic signaling, perhaps reducing orexinergic signaling gain by changes in synaptic plasticity, which continue to change orexin system responsiveness and promote further development of obesity. Animals with diet rich in cholesterol have seen their body weights increased significantly, thus developed obesity (De Moura e Dias et al., 2021). We know that cholesterol induces the hormone synthesis such as cortisol, the aldosteron, the testosteron and the oestrogens which are the sex hormones may be the increasing in cholesterol in the HCD rats would have contributed to increase the rate of these hormones and induce significantly increasing of body weight in rat fed with HCD (Fidele, Barama, Talla, & Dimo, 2017).

On the other hand, we observed in HCV group an increased in blood concentrations of cholesterol, TG, LDL and AIS and decreased HDL. Decreased GSH coupled with increased MDA concentrations in heart and liver indicate hyperlipidemia and compromised antioxidant status. The elevations in serum cholesterol, TG and LDL levels observed in this study were in agreement with those reported in several studies (Binmowyna Alfaris, Almnaizel, Alsayadi, & Abdo al sanea, 2021; Cunha, Ongaratto, Endres, & Barschak, 2021). It has been reported that high serum abnormally levels of TG and LDL are associated with an increased risk for atherosclerosis (Nwozo, Kasumu, & Oyinloye, 2015).

The current study showed that administration of virgin olive oil, the primary source of fat in the Mediterranean diet, reduced the AIS and liver lipids accumulation induced hypercholesterolemic rats. This study suggested that administration of virgin olive oil reduces excessive accumulation of lipids not only in the body but also in liver and thus loses body weight gain. Yeh, Cho, Hsieh, and Chiang (2018) confirmed that the ethyl acetate fraction of Chinese olive fruit extract significantly inhibited body weight gain, epididymal adipose tissue weight, and hepatic lipid accumulation via regulation of the expression of fatty acid transporter, lipogenesis, and fatty acid oxidation genes and proteins in C57BL/6 mice fed a 60% high-fat diet. Therefore, Chinese olive fruits may have the potential to improve the metabolic abnormalities associated with fatty liver under high fat challenge.

Wani, Rahiman, and Almaden (2017) demonstrated that a mice treated with olive oil showed decrease serum total cholesterol, serum triglycerides and low density lipoprotein (LDL) levels as compared animal group fed with high-fat diet. These results are in agreement with the previous studies using different animal model fed with high-fat diet and olive oil (Alhazza, 2007; Paoli, Cenci, & Grimaldi, 2011; Bigagli et al., 2019).

Khan, Iqbal, & Rashid (2017), premeditated effects of olive oil on lipid profile in diabetic patients, they studied upon 60 patients, and they found a significant reduction in serum cholesterol, TG levels in patients who used olive oil with statin drugs. Hernaez et al. (2015) assessed the effects of olive oil polyphenols on LDL concentrations. The consumption of olive oil polyphenols was significantly associated with a decrease in a total number of LDL particles. They explained the decrease in LDL concentrations through an improvement in the systemic oxidative status or by an increase in the gene expression of lipoprotein lipase (LPL). Three different mechanisms may be involved in this hypothesis. First, oxidative stress states are associated with increased LDL concentrations, especially due to an increased number of small LDL particles (Kotani, Tsuzaki, Taniguchi, & Sakane, 2012). An improved oxidative status due to the consumption of olive oil polyphenols may counteract increases in LDL concentrations by decreasing the number of small LDL particles. Second, increases in the expression of LPL may help the organism to decrease concentrations of TG-rich lipoproteins (e.g., LDLs), because LPL is the main enzyme involved in the removal of TGs from the blood and presents some LDL receptor activity (Reiner, 2018). Finally, improvements in general oxidative status have been associated with a better activity of LPL (Hernaez et al., 2015).

The resistance of lipoproteins to lipid peroxidation is modulated by both dietary fatty acids and antioxidants. Studies have shown that the intake of MUFA in the diet can modulate the susceptibility of LDL to oxidative modification. The susceptibility of LDL to oxidation depends on the PUFA/MUFA ratio, their oleic acid and antioxidant contents, as well as on the size of the LDL particle (Kuna & Achinna, 2013). Olive oil is a food which, besides high levels of MUFA, contains several minor components with biological properties.

The beneficial role of olive oil consumption is nowadays widely recognized. However, it is not clear whether its health effects are due to the presence of monounsaturated lipids and/or to the antioxidant fraction of microconstituents present in olive oil. At effective doses, omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid, have distinct effects on membrane structure, lipid dynamics, and rates of membrane lipid oxidation. EPA has been found to reduce markers of inflammation, cholesterol crystal formation, endothelial dysfunction, and oxidative modification of various ApoB-containing lipoprotein particles as well as increasing the functionality of HDL (Preston Mason, 2019).

Phenols, such as hydroxytyrosol and oleuropein, have shown antioxidant properties *in vitro* and *in vivo* studies (Posadino et al., 2021). However, low polyphenol content olive oil did not further modify HDL levels, while high polyphenol olive oil increased HDL-cholesterol concentration by almost 50% (Tsartsou, Proutsos, Castanas, & Kampa, 2019). On the other hand, sterols are bile acid sequestrants and acyl coenzyme A cholesterol acyltransferase activity (ACAT) inhibitors and its consumption leads to lower levels of plasma LDL cholesterol (Jesch & Carr, 2017).

The polyphenols of catechin, quercetin, and ethanol exert supportive effects against vascular disease, which can be explained by increased fibrinolytic activity and expression of the proteins involved in fibrinolytic system (Sedighi, Bahmani, Asgary, Beyranvand, & Rafieian-Kopaei, 2017).

We also observed the considerably reduce level of MDA and the considerably enhance concentration of the GSH in olive oil treated rats compared with HCD rats. Musumeci, Trovato, Imbesi and Castrogiovanni (2013) were studied the effects of dietary oleic acid on oxidative stress induced by exhaustive exercise in rat skeletal muscle, and were evaluated rats submitted to exhaustive exercise, inducing oxidative stress, and fed with an experimental chow enriched with oleic acid, present in high percentage in extra virgin olive oil (74-76%). They observed that, the parameters indicating oxidative stress such as hydroperoxides and thiobarbituric acid reactive substances decreased, parameters indicating antioxidant defenses of the body such as non-enzymatic antioxidant capacity and Hsp70 expression increased. This results support the concept that extra-virgin olive oil, rich in oleic acid, could represent an important protective factor against the uncontrolled production of ROS arising from physical effort.

Conclusion

The results of our study indicated that virgin olive oil exerted a marked hypolipidemic effect on the plasma lipids of rats fed an atherogenic diet. The plasma levels of TG, cholesterol and LDL decreased highly significantly after oral administration of virgin olive oil. These results indicated that virgin olive oil is an effective treatment to lower cholesterol levels and thus slow down the occurrence and development of CHD. On the other hand, the virgin olive oil reduced hepatic and heart tissues oxidative stress (MDA), which is characterized by elevation in the antioxidant glutathione levels. More clinical trials, preferably long-term studies, are necessary to evaluate and confirm the beneficial effects of olive oil and its compounds on atherosclerosis.

References

Alhazza, I. M. (2007). Antioxidant and hypolipidemic effects of olive oil in normal and diabetic male rats. *Saudi Journal of Biological Sciences, 14*(1), 69-74.

Amato, M., Bonomi, A., Laguzzi, F., Veglia, F., Tremoli, E., Werba, J. P., & Giroli, M. G. (2020). Overall dietary variety and adherence to the Mediterranean diet show additive protective effects against coronary heart disease. *Nutrition, Metabolism & Cardiovascular Diseases*, *30*, 1315-1321.

Balogun, E. A., & Adebayo, J. O. (2007). Effect of ethanolic extract of *Daniella Oliveri* leaves on some cardiovascular indices in rats. *Pharmacognosy Magazine, 3*(9), 16-20.

Bigagli, A., Toti, S., Lodovici, M., Giovannelli, L., Cinci, L., D'Ambrosio, M., &Luceri, C. (2019). Dietary Extra-virgin olive oil polyphenols do not attenuate colon inflammation in transgenic HLAB-27 rats but exert hypocholesterolemic effects through the modulation of HMGCR and PPAR-α gene expression in the liver. *Lifestyle Genomics,* 1-10. DOI: https://doi.org/10.1159/000495516

Binmowyna, M. N., Alfaris, N. A., Almnaizel, A. T., Alsayadi, M. M., & Abdo al sanea, E. (2021). Hypolipidemic and antioxidant effects of the juice and water seed extracts of two pomegranate species in high-cholesterol diet fed rats. *Food Science and Technology*, *41*(2), 732-740. DOI: https://doi.org/10.1590/fst.31220

Cunha, L. F., Ongaratto, M. A., Endres, M., & Barschak, A. G. (2021). Modeling hypercholesterolemia in rats using high cholesterol diet. *International Journal of Experimental Pathology*, *102*, 74–79.

De Moura e Dias, M., Aparecida dos Reis, S., Lopes da Conceição, L., Nogueira de Oliveira Sediyama, C. M., Pereira, S. S., Licursi de Oliveira, L., ... Milagro, F. I. (2021). Diet-induced obesity in animal models: points to consider and influence on metabolic markers. *Diabetology & Metabolic Syndrome*. DOI: https://doi.org/10.1186/s13098-021-00647-2

Diao, S. L., Sun, J. W., Ma, B. X., Li, X. M., & Wang, D. (2018). Influence of crocetin on high-cholesterol diet induced atherosclerosis in rats via anti-oxidant activity together with inhibition of inflammatory response and p38 MAPK signaling pathway. *Saudi Journal of Biological Sciences, 25*(3), 493-499. DOI: https://doi.org/10.1016/j.sjbs.2016.11.005

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics, 82*(1), 70-77. DOI: https://doi.org/10.1016/0003-9861(59)90090-6

Fidele, N., Barama, J., Talla, E., & Dimo, T. (2017). Hypolipidemic, antioxidant and antiatherosclerogenic effect of aqueous extract leaves of Cassia occidentalis Linn (Caesalpiniaceae) in diet-induced hypercholesterolemic rats. *BMC Complementary and Alternative Medicine, 17*, 1-11.DOI: https://doi.org/10.1186/s12906-017-1566-x

Folch, J., Lees, M., & Stanley, S. (1957). A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*(1), 479-503.

Hernaez, A. L., Remaley, A. T., Farras, M., Fernandez-Castillejo, S., Subirana, I., Schroder, H., ... Fito, M. (2015). Olive oil polyphenols decrease LDL concentrations and LDL atherogenicity in men in a randomized controlled trial1-3. *Journal of Nutrition, 145*(8), 1692-1697. DOI: https://doi.org/10.3945/jn.115.211557

Hopewell, J. C., Reith, C., & Armitage, J. (2014). Pharmacogenomics of statin therapy: any new insights in efficacy or safety? *Current Opinion in Lipidology, 25*(6), 438-445. DOI: https://doi.org/10.1097/MOL.0000000000000125

Jesch, E. D., & Carr, T. P. (2017). Food ingredients that inhibit cholesterol absorption. *Preventive Nutrition and Food Science*, *22*(2), 67-80. DOI: https://doi.org/10.3746/pnf.2017.22.2.67

Jose, J., Al-Tamimi, F. A., Helal, M. M., Jimmy, B., & Al Riyami, Q. (2014). Statin associated hepatic adverse effects: a retrospective review from a regional hospital in sultanate of Oman. *Oman Medical Journal, 29*(5), 351-357. DOI: https://doi.org/10.5001/omj.2014.93

Khan, T. M., Iqbal, S., & Rashid, M. A. (2017). Comparison of lipid lowering effect of extra virgin olive oil and Atorvastatin in dyslipidemia in type 2 diabetes mellitus. *Journal of Ayub Medical College Abbottabad, 29*(1), 83-86.

Kim, J. Y., & Shim, S. H. (2019). Medicinal herbs effective against atherosclerosis: classification according to mechanism of action. *Biomolecules & Therapeutics, 27*(3), 254-264. DOI: https://doi.org/10.4062/biomolther.2018.231

Kotani, K., Tsuzaki, K., Taniguchi, N., & Sakane, N. (2012). LDL particle size and reactive oxygen metabolites in dyslipidemic patients. *International Journal of Preventive Medicine, 3*(3), 160-166.

Kuna, A., & Achinna, P. (2013). Mono unsaturated fatty acids for CVD and diabetes: A healthy choice. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, *3*(3), 236-248.

Markin, A. M., Sobenin, I. A, Grechko, A.V., Zhang, D., & Orekhov, A.N. (2020). Cellular mechanisms of human atherogenesis: Focus on chronification of inflammation and mitochondrial mutations. *Frontiers in Pharmacoly*, *11*(642), 1-10. DOI: http:// doi.org/10.3389/fphar.2020.00642

Metwally, M. A. A., El-Gellal, A. M., & El-Sawaisi, S. M. (2009). Effects of Silymarin on lipid metabolism in rats. *World Applied Sciences Journal, 6*(12), 1634-1637.

Musumeci, G., Trovato, F. M., Imbesi, R., & Castrogiovanni, P. (2013). Effects of dietary extra-virgin olive oil on oxidative stress resulting from exhaustive exercise in rat skeletal muscle: a morphological study. *Acta Histochemica, 116*(1), 61-69. DOI: https://doi.org/10.1016/j.acthis.2013.05.006

Namazi, F., Shomali, T., Taghikhani, P., & Nazifi, S. (2018). Protective effect of *Urtica dioica* leaf hydro alcoholic extract against experimentally-induced atherosclerosis in rats. *Avicenna Journal of Phytomedicine, 8*(3), 254-262.

Nwozo, S. O., Kasumu, T. F., & Oyinloye, B. E. (2015). African Nutmeg (*Monodora Myristica*) lowers cholesteroland modulates lipid peroxidation in experimentally induced hypercholesterolemic male wistar rats. *International Journal of Biomedical Science*, *11*(2), 86-92.

Paoli, A., Cenci, L., & Grimaldi, K. A. (2011). Effect of ketogenic mediterranean diet with phyto extracts and low carbohydrates/high protein meals on weight, cardiovascular risk factors, body composition and diet compliance in Italian council employees. *Journal of Nutrition, 10*, 112- 120. DOI: https://doi.org/10.1186/1475-2891-10-112

Perez-Leighton, C. E., Butterick-Peterson, T. A., Billington, C. J., & Kotz, C. M. (2013). Role of orexin receptors in obesity: from cellular to behavioral evidence. *International Journal of Obesity, 37*(2), 167-174. DOI: https://doi.org/10.1038/ijo.2012.30

Posadino, A.M., Cossu, A., Giordo, R., Piscopo, A., Abdel-Rahman, W. M., Piga, A & Pintus, G. (2021). Antioxidant properties of olive mill wastewater polyphenolic extracts on human endothelial and vascular smooth muscle cells. *Foods*, *10*, 800. DOI: https://doi.org/10.3390/ foods10040800

Pourrajab, B., Sohouli, M. H., Amirinejad, A., Fatahi, S., Găman, M. A & Shidfar, F. (2021). The impact of rice bran oil consumption on the serum lipid profile in adults: a systematic review and meta-analysis of randomized controlled trials. *Critical Reviews in Food Science and Nutrition*. DOI: https://doi.org/10.1080/10408398.2021.1895062

Preston Mason, R. (2019). New insights into mechanisms of action for omega-3 fatty acids in atherothrombotic cardiovascular disease. *Current Atherosclerosis Reports, 21*, 1-11. DOI: https://doi.org/10.1007/s11883-019-0762-1

Reiner, Z. (2018). Triglyceride-Rich lipoproteins and novel targets for anti-atherosclerotic therapy. *Korean Circulation Journal*, *48*(12),1097-1119. DOI: https://doi.org/10.4070/kcj.2018.0343

Roman, G. C., Jackson, R. E., Gadhia, R., Roman, A. N., & Reis, J. (2019). Mediterranean diet: The role of long-chain v-3 fatty acids in fish; polyphenols in fruits, vegetables, cereals, coffee, tea, cacao and wine; probiotics and vitamins in prevention of stroke, age-related cognitive decline, and Alzheimer disease. *Revue Neurologique*, 1-18. DOI:https://doi.org/10.1016/j.neurol.2019.08.005

Sastre, J., Pallardó, F. V., Asuncion, J. G. de la, & Vina, J. (2000). Mitochondria, oxidative stress and aging. *Free Radical Research, 32*(3), 189-198. DOI: https://doi.org/10.1080/10715760000300201

Sedighi, M., Bahmani, M., Asgary, S., Beyranvand, F., & Rafieian-Kopaei, M. (2017). A review of plant-based compounds and medicinal plants effective on atherosclerosis. *Journal of Research in Medical Sciences, 15*, 22-30. DOI: https://doi.org/10.4103/1735-1995.202151

Subramani, C., Rajakkannu, A., Rathinam, A., Gaidhani, S., Raju, J., & Singh, D. V. K. (2017). Anti-atherosclerotic activity of root bark of *Premna integrifolia* Linn. In high fat diet induced atherosclerosis model rats. *Journal of Pharmaceutical Analysis, 7*(2), 123-128. DOI: https://doi.org/10.1016/j.jpha.2016.12.002

Tsartsou, E., Proutsos, N., Castanas, E., & Kampa, M. (2019). Network meta-analysis of metabolic effects of olive-oil in humans shows the importance of olive oil consumption with moderate polyphenol levels as part of the Mediterranean diet. *Frontiers in Nutrition, 6*(6), 1-11. DOI: https://doi.org/10.3389/fnut.2019.00006

Wani, F. A., Rahiman, S., &Almaden, A. R. H. A. (2017). Antioxidant modulating effect of Olive oil (*Olea europaea*) in the management of oxidative stress to combat obesity. Advanced. *Biomedical Research*, *8*, 67-74.

Yeh, Y. T., Cho, Y. Y., Hsieh, S. C., & Chiang, A. N. (2018). Chinese olive extract ameliorates hepatic lipid accumulation *in vitro* and *in vivo* by regulating lipid metabolism. *Scientific Reports, 8*(1), 1-13. DOI: https://doi.org/10.1038/s41598-018-19553-1